CONTENTS

PRESENTATION

309  Hermilo de la Cruz Yaínez

EDITORIAL

310  Fiftieth anniversary of the Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social
Fortino Solórzano Santos, Miguel Ángel Villasis Keever

REVIEW ARTICLES

313  Role of prolactin in the immune response
Francisco Blanco Favela, María Victoria Legorreta Haquet, Yunuen Rocio Huerta Villalobos, Karina Chávez Rueda, Eduardo Montoya Díaz, Luis Chávez, Edgar Zenteno Galindo
321  Docosahexaenoic acid and arachidonic acid in neonates: are they receiving a sufficient amount to meet their needs?
Mariela Bernabe-García, Raúl Villegas Silva, Mardia López Alarcón

RESEARCH ARTICLES

330  Coping strategies and their relation with depression and anxiety in pediatric residents in a third-level pediatric hospital
Ana Carolina Sepúlveda Vildásola, Ana Laura Romero Guerra, Leonel Jaramillo Villanueva
338  Frequency of infection and disease due to cytomegalovirus and risk of development in pediatric kidney transplant patients
Maria Antonieta Julián Núñez, Maria Guadalupe Miranda Novales, Eric Moisés Flores Ruiz, Ignacio Guerra Gallo, Fortino Solórzano Santos, José Guillermo Vázquez Rosales
349  Suckling behavior at 48 hours of life in low and normal birth weight infants and their growth at 28 days of life
Mario Enrique Rendón Macías, Héctor Domínguez Jiménez, Yolanda Aguilar Álvarez
357  Usefulness of C-reactive protein in the diagnosis of bacterial infection in the pediatric patient with cancer, fever and neutropenia
MJ Penagos Paniagua, Miguel Ángel Villasis Keever, Maria Guadalupe Miranda Novales, A Tapia Marcial, H Rivera Márquez, R Bernáldez Ríos, E López Aguilar, Fortino Solórzano Santos
364  Multimodal study of hand hygiene in a third-level pediatric hospital
Irma Zamudio-Lugo, Abigail Meza-Chávez, Yazmín Martínez-Sánchez, Guadalupe Miranda-Novales, Guadalupe Espinosa-Vital, Roberta Rodríguez-Sing
371  Pediatric and adolescent gynecology in a tertiary-level pediatric hospital: 15-years of experience
Abigail Hernández Cabeza, Juana Serret Montoya, Miguel Ángel Villasis Keever, Jesús Bonilla Rojas, Eulalia Garrido Magaña, Rocío Cárdenas Navarrete, Martha Elena Morales Castillo
376  Frequency and type of airway injury identified by bronchoscopic examination in newborns with prolonged endotracheal intubation in a neonatal intensive care unit
Heladia García, Hugo Ramírez San Juan, Jorge Ramirez Figueroa, Raúl Villegas Silva, Olivia Madrigal Muñiz
382 Reliability of nursing records of anthropometric measurements of patients in a tertiary pediatric hospital
Miguel Ángel Villasis Keever, Norma Andrea Arias Villa, María Guadalupe Cedillo Rosas, Ivonne Hernández Luna, Karla Cristina Emiliano Aceves, Vianey Mora Gutiérrez, Martha Alicia Sánchez Ramírez, Jessi Nallely Zurita Cruz

388 Steroid-resistant nephrotic syndrome: 15 years experience from the Hospital de Pediatría, Centro Médico Nacional Siglo XXI
María Alejandra Aguilar Kitsu, Claudia del Carmen Zepeda Martínez, María del Pilar Ibarra Cazares, Juana Lorena Sánchez Barbosa, Ramiro Alejandro Luna Sánchez María Leticia, Mendoza Guevara, Karina Diaz de León, José Manuel Ubillo
SUBSCRIPTIONS

In Mexico: $500 pesos
Outside Mexico: $60 USD

FORMS OF PAYMENT

Cash
Direct at the cashier
Hospital Infantil de México Federico Gómez
Received
Editing Department Médicas
Edificio Mundet, tercer piso
Dr. Márquez 162, Col. Doctores
06720 Cuauhtémoc, Mexico City
Hours: M-F: 8 am - 3 pm

Bank deposit
Banorte: 0102801543
Send copy of the deposit by FAX:
(55) 57 61 89 28

Electronic bank transfer
Banorte
Routing number: 072180001028015432
“Fondo de Ediciones Médicas”

Check
Send by mail to:
Hospital Infantil de México Federico Gómez

CONDITIONS:

Issues will be delivered or mailed to all subscribers either directly or by mail. For mailed issues, please include the following information:
Name
Address
Telephone
E-mail

FOR QUESTIONS OR ANY ADDITIONAL INFORMATION:

Tel./Fax: (55) 57 61 89 28
Email: bolmedhim@yahoo.com.mx

The Pediatric Journal with the highest diffusion in Mexico

More than 65 years of uninterrupted publication. Six issues each year with more than 70 articles authored by national and international investigators with the most relevant themes
Boletín Médico del Hospital Infantil de México

Federico Gómez Santos †
Founder

José Alberto García Aranda
General Director

Onofre Muñoz Hernández
Associate Director

Gonzalo Gutiérrez
Editor

María G. Campos Lara
Executive Editor

Ricardo Viguri Uribe
Associate Editor and Administrator

Sharon Morey
Associate Editor

Julia Segura Uribe
Adjunct Editor

EDITORIAL COMMITTEE

BIOMEDICAL
Jesús Kumate Rodríguez
Pedro Valencia Mayoral

PUBLIC HEALTH
Sonia Fernández Cantón
Hortensia Reyes Morales

PUBLIC HEALTH PEDIATRIC THEMES
Luis Jasso Gutiérrez
Luis Velásquez Jones

HEALTH EDUCATION AND CLINICAL ETHICS
Jaime Nieto Zermeno
Juan José Luis Sierra Monge

CLINICAL
Blanca Estela del Río Navarro
Fortino Solórzano Santos

CLINICAL EPIDEMIOLOGY
Juan Garduño Espinosa
Miguel Ángel Villasis

CLINICAL CASES
Salvador Villalpando Carrión

CLINICOPATHOLOGICAL CASES
Stanislaw Sadowinski Pine

1 Fundación IMSS
2 Hospital Infantil de México Federico Gómez
3 Hospital de Pediatria, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social
4 Instituto Nacional de Salud Pública, Secretaría de Salud
5 Dirección de Información Epidemiológica, Dirección General de Epidemiología, Secretaría de Salud
EDITORIAL BOARD

JOSÉ LUIS ARREDONDO GARCÍA
MANUEL BAEZA BACAB
EDUARDO BANCALERI
ALESSANDRA CARNEVALE CANTONI
ALDO CASTAÑEDA
LECTICIA CASTILLO

FRANCISCO CIGARROA
ALEJANDRO CRAVIOTO QUINTANA
BLANCA ESTELA DEL RÍO NAVARRO
ALFONSO DELGADO RUBIO
ARTURO FAJARDO GUTIÉRREZ
SAMUEL FLORES HUERTA
CARLOS FRANCO PAREDES
SARA HUERTA YEPEZ
FIMA LIFSHTIZ
GABRIEL MANJARREZ
HOMERO MARTÍNEZ SALGADO
MARA MÉDEIROS
JUAN PABLO MÉNDEZ BLANCO

GUADALUPE MIRANDA NOVALES
VERÓNICA MORÁN BARROSO
ÁNGEL NOGALES ESPERT
SAMUEL NURKO
MIGUEL O’RYAN
ALBERTO PEÑA
FRANCISCO J. PUGA MUÑOZURI
GUILLERMO RAMÓN
VESTA RICHARDSON LÓPEZ COLLADA

FABIO SALAMANCA GÓMEZ
EDUARDO SALAZAR LINDO
NORBERTO SOTELO CRUZ
ALEJANDRO SWEET CORDERO
GUSTAVO VARELA FASCIINETTO
ARTURO VARGAS ORIGEL
EDGAR VÁSQUEZ GARIBAY
FEDERICO RAÚL VELÁZQUEZ
ALBERTO VILLASEÑOR SIERRA

INSTITUTO NACIONAL DE PEDIATRÍA
CENTRO MÉDICO DE LAS AMÉRICAS
HOLTZ CHILDREN´S HOSPITAL
INSTITUTO NACIONAL DE MEDICINA GENÓMICA
UNIDAD DE CIRUGÍA CARDIOVASCULAR DE GUATEMALA
CHILDREN´S MEDICAL CENTER,
UNIVERSITY OF TEXAS SOUTHWESTERN

University Hospital
ORESPES S.A. DE C.V.
Hospital Infantil de México Federico Gómez
Hospital Universitario Madrid Sanchinarro
Centro Médico Nacional S. XXI, IMSS
Hospital Infantil de México Federico Gómez
Emory University Hospital
Hospital Infantil de México Federico Gómez
Centro Médico Nacional S. XXI, IMSS
Hospital Infantil de México Federico Gómez
Hospital Infantil de México Federico Gómez
Centro Médico Nacional S. XXI, IMSS
Hospital Infantil de México Federico Gómez
Hospital Universitario Reina Sofía
Children´s Hospital Boston
Universidad de Chile
Cincinnati Children´s Hospital
Mayo Clinic
Hospital Infantil de México Federico Gómez
Centro Nacional de Salud para la Infancia y la Adolescencia
Centro Médico Nacional S. XXI, IMSS
DS-CONSULT S.A.C.
Escuela de Medicina, Universidad de Sonora
Stanford University School of Medicine
Hospital Infantil de México Federico Gómez
Facultad de Medicina, Universidad de Guanajuato
Instituto de Nutrición Humana
Centro Médico Nacional S. XXI, IMSS
Centro de Investigaciones Biomédicas de Occidente

MÉXICO D.F., MÉXICO
MÉRIDA, YUCATÁN, MÉXICO
MIAMI, FLORIDA, U. S.
MÉXICO D.F., MÉXICO
GUATEMALA, GUATEMALA
DALLAS, TEXAS, U. S.
SAN ANTONIO, TEXAS, U. S.
MÉXICO D.F., MÉXICO
MÉXICO D.F., MÉXICO
MADRID, ESPAÑA
MÉXICO D.F., MÉXICO
DIVA, GEORGIA, U. S.
MÉXICO D.F., MÉXICO
ST. BARBARA, CALIFORNIA, U. S.
MÉXICO D.F., MÉXICO
MÉXICO D.F., MÉXICO
CÓRDOBA, ESPAÑA
BOSTON, MASSACHUSETTS, U. S.
SANTIAGO DE CHILE, CHILE
CINCINNATI, OHIO, U. S.
ROCHESTER, MINNESOTA, U. S.
MÉXICO D.F., MÉXICO
MÉXICO D.F., MÉXICO
LIMA, PERÚ
HERMOSILLO, SONORA, MÉXICO
STANFORD, CALIFORNIA, U. S.
MÉXICO D.F., MÉXICO
LEÓN, GUANAJUATO, MÉXICO
GUADALAJARA, JALISCO, MÉXICO
MÉXICO D.F., MÉXICO
GUADALAJARA, JALISCO, MÉXICO
Since its foundation almost 50 years ago, the Medical Unit of High Specialty (UMAE) of the Pediatric Hospital, Centro Medico Nacional Siglo XXI, Mexico City has been dedicated to provide medical care to pediatric patients with the highest quality of care standards. This has been largely possible due to the systematized evaluation of each one of the components of the process of care provided to all patients who are treated in the UMAE.

Throughout the years, results obtained in regard to the care of children, response to complications, scrutiny of possible pathophysiological mechanisms and proposed changes in the diagnostic and therapeutic processes have been presented to the national and international medical community through innumerable publications elaborated by the hospital’s medical staff members.

In this special edition, due to the vision of the Boletín Médico del Hospital Infantil de México, it is my pleasure to present to our colleagues a collection of 11 articles, representing only a sample of the current scientific productivity in the UMAE Pediatric Hospital.

Dr. Hermilo De La Cruz Yáñez
Director General
Unidad Médica de Alta Especialidad Hospital de Pediatria
Centro Médico Nacional Siglo XXI
Instituto Mexicano del Seguro Social
Mexico, D.F., Mexico
E-mail: hermilo.delacruz@imss.gob.mx
Fiftieth anniversary of the Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social
Fortino Solórzano Santos, Miguel Ángel Villasis Keever

In May 1961, the network of hospitals that comprise Centro Médico Nacional (CMN), now Siglo XXI (21st Century) of the Instituto Mexicano del Seguro Social (IMSS) was inaugurated. During this time, the General Hospital (presently the Specialty Hospital), Cardiology Hospital, Trauma and Orthopedics Hospital, Gynecology and Obstetrics Hospital, Oncology Hospital and Pediatric Hospital opened their doors. From the time of their opening, the “building blocks” of the different hospitals that comprise this Medical Center have been care (medical care), education (training of nurses, nutritionists, general practitioners and specialists) and research, which continue to be promoted up to present times.

As a way to recognize what these 50 years of medical history have meant at the IMSS Pediatric Hospital, in this special issue of Boletín Médico del Hospital Infantil de México (BMHIM) a series of review and original research articles have been brought together, reflecting the multi- and interdisciplinary work that is currently being developed at the Pediatrics Hospital, Centro Médico Nacional Siglo XXI. Throughout history, the Pediatric Hospital has generated numerous medical publications, with research focusing on priority health problems according to the country’s socioeconomic conditions. There is evidence of contributions to pediatrics from the institutional founders up to those developed by various clinical and basic researchers in current times.1-23

In this special issue, different aspects of research carried out in this hospital during recent years are presented.24-34 These articles describe, in part, the process of care provided in the Pediatric Hospital, both according to the type of patients served as well as their biological age. Thus, in the area of neonatology, nutritional problems in the newborn and the need for special support as well as the impact of feeding technique used are demonstrated.28 Technological advances have enabled the survival of preterm and low birthweight infants who require mechanical ventilation and prolonged intubation as part of their management. As a result of these conditions, we present an analysis of airway injury associated with this treatment.32

Furthermore, the current characteristics of the Pediatric Hospital have encouraged a higher level of patient care for those with different degrees of immunosuppression due to baseline health conditions. Patients subjected to transplants require the use of immunosuppressants for their management. These increase the risk of acquiring opportunistic infections, among which is cytomegalovirus infection. In this issue we analyze the frequency of this infection in patients receiving kidney transplants and its relation to a variety of risk factors.27 On the other hand, it is often that hematology-oncology patients have varying degrees of neutropenia during certain phases of treatment, particularly...
with chemotherapy. This also renders these patients susceptible to various infectious processes. In clinical practice there is often difficulty in distinguishing—in the case of a neutropenic febrile patient—cases of bacterial infection and cases without an infectious problem. Determination of C-reactive protein is used as an auxiliary test for suspected bacterial infection in accordance with serum levels presented by the patient. Penagos et al. sought the best cutoff value to identify patients who present with an underlying infectious process. In this context, the immune process has many variants, a reason for which it was considered of interest to include a review on the participation of prolactin in modulating the immune response.

The pathologies observed in pediatric patients, both in young children and adolescents, involve different diseases that can result in chronic conditions, among which are kidney diseases. As an example, we analyze the evolution of a group of patients with nephrotic syndrome undergoing different treatment regimens, along with their prognoses achieved over the years. In the context of this age group, female patients are always included who, when physiological growth changes are achieved, reach the time of sexual maturation. Many of these girls with chronic illnesses suffer different hormonal changes, which coupled with the underlying disease, generate events that require medical support. The Medical Unit of High Speciality (UMAE) of the Pediatric Hospital has established a clinic for gynecological care, whose experience is addressed in one of the articles.

In the healthcare environment, there are certain processes that sometimes are not validated as often as they should be and are part of quality of care provided to patients. Therefore, articles that addressed these issues were included. One of these articles describes the reliability of anthropomorphic measurements carried out by the nursing staff, measurements that are essential to the care of pediatric patients. The second corresponds to the results of monitoring the practice of hand washing as a fundamental measure to control nosocomial infections and represents one of the International Goals of Patient Safety (which ideally should be met in 100% of cases). Last but not least, a great deal of care in hospitals is according to the responsibility of medical residents. Within the different training stages, medical residents are subjected to academic studies, working time, and family and economic considerations, all types of activities that generate different degrees of stress which, in turn, result in varying degrees of anxiety or depression. Sepulveda et al. presented the coping mechanisms most frequently used by a group of Pediatric Hospital residents, manifesting some of the needs that sometimes require medical attention by the residents themselves.

It is expected that the readers, through the various articles, will become familiar with part of the work that is performed daily in the UMAE Pediatric Hospital and with the experience of the investigations published will continue to contribute to improving pediatric patient care.

This special issue of BMHIM represents a “gift” for the celebration of the 50th anniversary of Centro Medical Nacional Siglo XXI, Mexican Institute of Social Security.

REFERENCES

Role of prolactin in the immune response
Francisco Blanco-Favela, María Victoria Legorreta-Haquet, Yunuem Rocío Huerta-Villalobos, Karina Chávez-Rueda, Eduardo Montoya-Díaz, Luis Chávez-Sánchez, Edgar Zenteno-Galindo

ABSTRACT
Evidence exists about the relationship between the immune system and the endocrine system through communication of multiple factors such as cytokines, neuropeptides, neurotransmitters and hormones. Among the hormones, prolactin (PRL) has been shown to participate in the innate and adaptive immune response. In addition to being produced by the pituitary gland, PRL is also produced and secreted by cells of the immune system. The aim of this review is to update information about the involvement of PRL secreted by immune system cells in the immune response.

Key words: Prolactin, PRL-R, immune response, Th1, Treg and Teff cells.

INTRODUCTION
Prolactin (PRL) is a polypeptide hormone with multiple functions that include maintenance of pregnancy, breast development, fluid homeostasis and immunomodulation. Regulation of the differentiation of secretory glands, lactation and the formation and activity of corpus luteum. PRL is synthesized and secreted by anterior pituitary cells (lactotrophs). Secretion of the stimulus can be induced by various factors such as sucking, direct stimulation of the skin of the areola, stress, or substances such as serotonin, cholecystokinin, angiotensin II, thyrotropin-releasing hormone and vasoactive intestinal peptide and are inhibited by dopamine and opiates. PRL has been associated with >300 different biological functions classified into five categories: 1) reproduction, 2) osmoregulation, 3) growth and development, 4) carbohydrate and lipid metabolism and 5) immunoregulation.

In the immune system, PRL has been implicated in T lymphocyte proliferation, protection against apoptosis and cell survival. It acts as an adaptive molecule to stress against inflammatory mediators important in maintaining homeostasis of the immunological system and participates in IgA transit through the cellular epithelium during mammary gland development.

Furthermore, it has been shown that synthesis and secretion of this hormone is not restricted to the pituitary but that cells of other organs and tissues have this capability, including cells of the immune system.

Prolactin
PRL is a globular, single-chain protein comprised of 199 amino acids (aa) and three intramolecular disulfide bridges (Cys4–Cys11, Cys58–Cys174 and Cys191–Cys199). Fifty percent of the amino acids in the chain form a secondary α-helix structure (Figure 1). The primary form of PRL, with a 23-kDa molecular weight, has been found in pituitary and serum. Various isoforms have been described resulting from posttranscriptional posttranslational modifications or chemical modifications in its amino acid chain: glycated (25 kDa) that exhibits a reduced biological activity, macroprolactin (big-big PRL >100 kDa) and big PRL (40–60 kDa), two isoforms of high molecular weight ari-
singing from its dimerization or polymerization\textsuperscript{21} or its binding with other proteins such as antibodies (150 kDa)\textsuperscript{22} and 16 kDa (potent angiolytic factor), which is a product of the enzymatic degradation of 23-kDa PRL.\textsuperscript{8,23}

According to its genetic, structural and functional characteristics, PRL belongs to the same family as growth hormone and placental lactogen, which arise from the same ancestral gene. The gene that codes for PRL is located on the short arm of chromosome 6 and is composed of five exons and four introns with sizes $\sim$10 kb.\textsuperscript{12,24-27} Expression of this gene has been identified in several regions of the brain, myometrium, thymus, spleen, bone marrow, mammary epithelial cells, endometrium, some tumor cell lines, immune cells (T and B lymphocytes), fibroblasts and sweat glands.\textsuperscript{5,12,17,28} PRL exerts various actions and may depend on its structural polymorphism as well as the wide distribution of its receptor.\textsuperscript{15}

**PRL Receptor**

PRL receptor (PRL-R) is a transmembrane protein whose gene is located on chromosome 5. Unlike the prolactin gene, which codifies for only one protein, PRL-R codifies for three different isoforms. These isoforms differ in size (short, intermediate, and long) and composition of the intracellular portion (Figure 2). They exhibit an identical extracellular domain of $\sim$200 aa. The transmembrane domain has 24 aa, whereas the intracellular domain has different sizes and compositions depending on the receptor isoform: the long isoform (598 aa in length), the intermediate isoform (325 aa) and two small isoforms (352 and 264 aa each).\textsuperscript{29} In this portion there are two preserved regions referred to as box 1 and box 2. The region closest to the membrane (box 1) has a proline-rich zone, whereas box 2 is a less-preserved region that is lost in a short isoform. The short and long isoforms of PRL-R are differentially expressed in various tissues, suggesting effects and activation of different routing signals.\textsuperscript{30} The long isoform is common in humans. A negative regulation of the function of the long isoform has been attributed to the short isoforms.\textsuperscript{29} Pituitary PRL may participate in regulation of the prolactin receptor gene in cells of the human immunological system.

Expression of the PRL receptor gene of lymphocytes was significantly suppressed in breastfeeding mothers. This new evidence suggests that the level of the PRL receptor of the circulating lymphocytes may be negatively regulated by elevated levels of serum PRL and that the PRL secreted by the pituitary may regulate expression of the PRL receptor of the immune cells, specifically during the postpartum period.

These data support the evidence that there is a role for PRL secreted by the pituitary in the immunological system under physiological circumstances.\textsuperscript{31} The tertiary structure of the receptor determined by crystallography shows that the extracellular domains have seven $\beta$-folded antiparallel chains (Figure 3).\textsuperscript{12,32}
Role of prolactin in the immune response

Receptor expression in brain cells, retina, cartilage, skin, lung, heart, pancreas, liver, spleen, thymus, intestinal tract, kidney, reproductive system, lymphocytes (B and T), macrophages, etc. has been demonstrated.\textsuperscript{12,33-35} PRL receptor together with IL2 (β and γ chains), IL3, IL4, IL6, IL7, IL9, IL12, IL15, GM-CSF, G-CSF, EPO, LIF, and growth hormone belongs to the superfamily of hematopoietic cytokine receptors.\textsuperscript{19,35-37}

Junction of PRL with its receptors induces tyrosine phosphorylation (Tyr) of different intracellular proteins including the receptor. The intracellular region closest to the membrane was constitutively associated with JAK2, which is phosphorylated 1 min after the interaction of PRL–PRL-R (Figure 4). The JAK2 protein phosphorylates to STAT, which presents five different domains: a) DNA binding, b) similar to SH2, c) similar to SH3, d) amine terminal and e) carboxyl terminal. Phosphorylated Tyr of JAK2 binds to the SH2 domain of STAT, which is phosphorylated by the association of PRL-R–JAK2. Once phosphorylated, STAT dissociates from the receptor, forming a homo- or heterodimer, which is translocated to the nucleus, activating the DNA binding domain. The sequence that recognizes the homo- or heterodimer of STAT1, STAT3 and STAT5 in the nucleus is a sequence that activates IFN-γ (GAS) and consists of a palindromic sequence (TTCxxxGAA) present in different promotors.\textsuperscript{8,12,34,38-40} Using this route, transcription of genes key in the development of the Th1 response is activated, such as the T-bet factor, which increases at low doses of PRL and is inhibited at high doses in CD4+ T lymphocytes.\textsuperscript{41}

Other protein tyrosine kinases (PTKs) are activated by PRL stimulation including Fyn, Src, Ras and Raf, and serine-threonine kinases such as ZAP-70, PI3, Akt, MAPK, JNK and PKC. The coordination of a parallel kinase cascade with the JAK/STAT signaling pathway may determine the expression pattern of genes from various tissues and cells in response to PRL (Figure 5). The pleiotropic actions of PRL related with cellular proliferation, differentiation, apoptosis or cell survival depend on the interactions among these parallel kinase cascades.\textsuperscript{13}

PRL and the Immune System

The relationship between PRL and the immune system was demonstrated in 1930 when Smith observed thymus

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Structure of the PRL receptor (PRL-R). The tertiary structure of the PRL-R has a binding site and a folded structure in antiparallel.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Activation mechanism of PRL-R by PRL-induced dimerization.}
\end{figure}
atrophy in hypophysectomized rats.\(^4\)\(^2\) Subsequently, Nagy and Bercy published their work on immunodeficiency in hypophysectomized rats where administration of PRL, growth hormone and placental lactogen restored immunological activity.\(^4\)\(^3\) In 1983 similar experiments were performed using bromocriptine (D2 dopamine agonist) to selectively inhibit PRL secretion, finding results similar to the previous study. Decrease in both cellular and humoral immune response is reestablished upon discontinuation of bromocriptine.\(^4\)\(^4\) It has also been found that the immune system is able to regulate PRL secretion.\(^1\)\(^9\) Cytokines IL1, IL6 and TNF\(\alpha\) can act as endocrine regulators in release of pituitary PRL.\(^4\)\(^5\),\(^4\)\(^6\)

In 1987 it was demonstrated that the culture supernatants from murine splenocytes activated with concanavalin A (Con A) induced proliferation in Nb2 cells (dependent on PRL) and their effect was reversed in the presence of anti-PRL antibodies.\(^7\) This suggested that lymphocytes could synthesize a protein with biological activity similar to pituitary PRL. This was demonstrated with in situ hybridization analysis, which revealed expression of messenger RNA (mRNA) of PRL in different lymphatic tissues.\(^1\)\(^5\),\(^4\)\(^7\) Using Northern blot on a lymphoblastoid B cell line, the presence of PRL mRNA was also demonstrated and, subsequently by RT-PCR technique, it was found that messenger PRL is not only expressed in lymphoid cell lines but also in normal lymphocyte cells such as thymocytes and human peripheral blood mononuclear cells.\(^1\)\(^5\)

Using immunofluorescence, it was observed that the PRL receptor is expressed on NK CD56+ uterine cells in situ and their expression was demonstrated in NK CD56+ decidual cells using RT-PCR and Western blot. In addition to phosphorylation of ERK1 and 2 on NK CD56+ cells in cultures with added PRL, it has been suggested that PRL stimulates ERK in multiple cellular compartments of human endometrium, identifying NK CD56+ uterine cells as a new target site for PRL.\(^4\)\(^8\)

PRL receptor expression in human NK cells is a clear indication that these cells are potentially responsive to stimulation with PRL, a fact confirmed only in rats. In humans, PRL increases IL-2R\(\alpha\) expression on the cell surface and on mRNA expression of IL-2, indicating that PRL overregulates NK cell function, favoring the feedback between IL-2 and its receptor. Similar results have been reported regarding IL-15 and IL-15R.\(^4\)\(^9\) PRL is also involved in cell differentiation and expression of various factors during differentiation of NK cells. It is expressed and required for a response of LAK cells. These data indicate a possible role for PRL in the modulation of NK cell function in humans.\(^5\)\(^0\)

Administration of recombinant PRL in mice with bone marrow transplant induces an increase in lymphopoiesis.\(^5\)\(^1\) In knockout mice, no defects are found in the production of lymphocytes with PRL and its receptors. However, the number of absolute B lymphocytes and its precursors are found to be mildly decreased, which demonstrates the participation of PRL in lymphopoiesis.\(^4\)\(^3\)\(^2\) Also, in cellular lines of Pro-B lymphocytes (B220\(^{low}\)CD43+) transected with PRL-R, as well as in mice Pro-B lymphocytes, PRL increases differentiation towards Pre-B (B220\(^{+}\)CD43-) lymphocytes.\(^5\)\(^3\) With respect to the co-stimulating molecular expression, no differences have been reported between expression of CD40 and CD86 lymphocytes from healthy individuals or patients with hyperprolactinemia.\(^5\)\(^4\)

In murine splenocytes it has been found that PRL increases viability and the stimulating capacity of dendritic cells (DC) and overregulates expression of MHC-II and CD40 while decreasing CD54 levels on DC. In addition, PRL decreases the levels of NF-xB p65 and endocytic capacity of DC and increases secretion of IL-6, IL-10, IL-12 and TNF-\(\alpha\) in these cells. This suggests that PRL may regulate the immune response, either physiological or pathological, through changes in viability of the phenotypic, endocytic and stimulatory capacity of DC as well as cytokine expression.\(^5\)\(^5\)

---

**Figure 5.** Schematic representation of the long and short PRL-R signaling in rats.
PRL increases expression of CD69 and CD25 in mononuclear cells and intervenes in the expression of CD69 and CD154 molecules on T CD4+ lymphocytes. When activated with PMA, autocrine PRL is blocked with anti-PRL antibody, molecular expression is markedly decreased as well as secretion of IL2 and IFN-γ. Moreover, mononuclear cells activated with LPS plus PRL increase secretion of IL12 and TNFα. Similarly, cytokines affect the mRNA expression of PRL in T lymphocytes and IL4 and IL1β and reduce messenger expression.

On blocking PRL specifically with an anti-PRL antibody, proliferation of mononuclear cells and lymphocytes activated with Con A are inhibited, as well as B lymphocytes activated with LPS and secretion of cytokines such as IL2, which act as a growth factor in Nb2 cells. The addition of exogenous PRL, and not of growth hormone, prevents the inhibitory action of the antibody in lymphocyte cultures. Also, in studies where cellular lines dependent on IL2 and IL4 were used, it was demonstrated that the same anti-PRL antibody inhibited the proliferative response towards these cytokines and the addition of PRL combined with IL2, phytohemagglutinin or Con A stimulated the proliferation of T and B lymphocytes and NK cells maintained in culture. In granulocytes, lymphocytes and endometrial cells, PRL induces gene transcription of the interferon regulating factor (IRF-I), which is an important regulator of the differentiation and maturation of T and B lymphocytes. It also regulates the synthesis of nitric oxide synthase (iNOS), mediating the immune response and inflammation. PRL also stimulates IL2 synthesis and its receptor in splenocytes and thymocytes in addition to collaborating in the actions of IL2 and IL12 stimulating IFN-γ synthesis in T lymphocytes and NK cells. Some studies suggested that PRL participates in the hematopoietic activity and development and maintenance of bone mass. On the other hand, PRL prevents apoptosis induced by nitric oxide and dexamethasone in the Nb2 cellular line and modulates expression of the genes implicated in apoptosis (bax and bcl-2) as well as caspase-3 activation. Studies performed in animal models show that PRL induces the expression of the IL2 receptor in lymphocytes of ovariectomized rats. It has also been reported that PRL favors the survival and differentiation of lymphoid progenitors and demonstrates the importance of PRL in activation and proliferation of lymphocytes.

Autocrine regulation of PRL signaling in the development and activation of T lymphocytes has been demonstrated, which reinforces the hypothesis of a mechanism by which autocrine PRL participates in immunoregulation of lymphocytes modulating the expression of costimulatory molecules and cytokines. For example, it is known that PRL is produced and secreted by cells of the immune system and acts immediately after the first signal of autocrine activation. Secretion of IL-2, IFN-γ and expression of CD69 and CD154 molecules partially depend on PRL.

Our group reported the involvement of PRL in the suppressive function of regulator T lymphocytes. Regulatory T lymphocyte (Treg) cells are CD4+ T cells that arise during thymus development and have suppressor characteristics. Development of T cells in the thymus is governed by two main processes: 1) positive selection, which is particularly critical in the development of T αβ lymphocytes that recognize the antigen associated with MHC molecules and ensures that the T cell is capable of responding, and 2) negative selection where T lymphocytes with highly attuned receptor reactions are eliminated and autoimmune reactions are prevented. In the periphery, stringent signaling requirements intrinsically regulate activation of T cells, whereas extrinsic regulation is carried out by Treg lymphocytes. These cells have been implicated in controlling the initial events of activation, proliferation, and differentiation and effector function of the target cells, which may be more susceptible or resistant to various regulatory mechanisms of Treg cells, depending on their functional stage or activation.

Almost all scientific publications reporting on natural as well as induced Treg lymphocytes speculate about its possible clinical applications. For example, if there is an excessive immunological response, such as in the case of autoimmune disease, asthma, allergy, transplant rejection or certain cases of premature fetal loss, it is believed that increasing the quantity or function of the Treg lymphocytes would have a beneficial effect. To remove or reduce Treg cells also provokes an effective humoral immunity in non-responding animals and an increase in the antimicrobial immunity in chronic infections, favoring the elimination of microbes and tumors. For this reason it would be interesting to explore the role of PRL in the regulatory phenomenon between effector and regulatory T cells. It has been recently demonstrated for the first time that ex-
pression of the PRL receptor is constitutive in regulatory T cells, unlike T effector cells, that require stimulation with anti-CD3/CD28 to induce the expression of the PRL receptor. It has also been demonstrated that PRL inhibits the function of regulatory T cells apparently through the induction of a secretory profile of type Th1 cytokines, which is in agreement with the findings reported by other authors regarding PRL favoring the secretion of Th1 cytokines in vivo and in vitro.

The ability to perform various actions relevant to immune system function has resulted in PRL being considered as a cytokine, a protein distinguished for sharing diverse characteristics such as participation in the immune response, being synthesized by multiple cell types, pleiotropic, with redundant effects and acting together with other cytokines to produce synergistic additive effects or to mutually antagonize their actions. PRL meets many of these characteristics. PRL and its receptor are important modulators of the immune response and may participate in its regulation.2

Several studies have shown that PRL is involved in the immune response by interacting with its receptor whose presence has been described in various cells of the immune system. PRL influences in the proliferation, activation and secretion of cytokines for T cells. In murine models it has been shown that PRL plays a relevant role in B-cell lymphopoiesis.

Similarly, the differential expression of the PRL receptor in subpopulations of T-cells has been demonstrated where regulatory T cells (CD4+CD25hiCD127low/-) constitutively express the PRI receptor, unlike effector T-cells (CD4+CD25CD127+) that require prior stimulus, via CD3/CD28 in order to induce the expression of the receptor. PRL also deregulates the suppressor function of Treg cells, probably by the induction of a secretory profile of type Th1 cytokines. The former would help to partially explain the relationship between hyperprolactenemia and activity of the disease in patients with SLE where high PRL levels may affect the suppressor function of Treg cells and favor a profile of type Th1 cytokine secretion, perpetuating the autoimmune response and exacerbating disease activity. Another possible application of this knowledge may be induction of hyperprolactinemia to control some infectious diseases. However, it requires delving into the mechanisms of action on the immune response carried out by PRL.

**Acknowledgments**

This study was partially funded by the Fondo de Investigación en Salud (Instituto Mexicano del Seguro Social) (registry number FIS-IMSS-PROT-G10-834) and by Consejo Nacional de Ciencia y Tecnología (CONACYT-113815).

**REFERENCES**

42. Smith PE. The effect of hypophysectomy upon the involution of the thymus in the rat. Anatomi Rec 1930;47:119-129.


Docosahexaenoic acid and arachidonic acid in neonates: are they receiving a sufficient amount to meet their needs?

Mariela Bernabe-García,1 Raúl Villegas-Silva,2 Mardia López-Alarcón1

ABSTRACT

In this review we discuss the physiological bases of n-6 and n-3 polyunsaturated fatty acids (PUFAs) and their end products: arachidonic acid (AA) and docosahexaenoic acid (DHA), respectively, to identify their importance in the fetal stage such as critical structural functions at 40 weeks of gestation. PUFA deficit is related to pathologies such as retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia (BPD), among others, in preterm infants who did not achieve adequate accretion. In addition, studies evaluating the effect of supplementation with different concentrations of PUFAs on neurological and visual function and growth in neonates are analyzed. We also address the needs of DHA and AA at this stage of life and compare the enteral intake achieved by human milk feeding and the different formulas for preterm and term infants.

DHA concentration in breast milk is highly variable and its supply may be insufficient in neonates. Preterm infant formulas can meet international recommendations of DHA and AA issued by different organizations but, due to preterm birth, these infants have scarce tissue reserves but increased requirements for these fatty acids. Thus, enteral intake using current supplemental formula feeding appears to be insufficient. The final recommendation is to feed neonates with human milk by offering information to mothers regarding food sources with high DHA content, especially in the case of preterm babies.

Key words: DHA, AA, neonates, LC-PUFA recommendations, nutritional content, human milk, LC-PUFA supplements, Mexico.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are characterized as having at least one double bond in the third carbon (n-3) or the sixth carbon (n-6) family, counting from the terminal methyl group of the hydrocarbon chain. Linoleic acid (LA) and alpha-linolenic acid (ALA) are considered to be essential fatty acids (EFAs) because they cannot be synthesized by mammalian cells in the absence of D-15 and D-12 desaturases. ALA and LA must be obtained from external sources, either dietary or through supplementation.1,2

ALA and LA are precursors of other fatty acids (FAs) from the n-3 and n-6 families, respectively, which are converted into their long-chain metabolites through elongation and desaturation. These metabolites, collectively called long-chain (LC)-PUFAs, are characterized by containing ≥20 carbon atoms and ≥3 double bonds. LC-PUFAs derived from LA, arachidonic acid (AA, 20:4 n-6), predominate in cell membranes in mammals and in human milk. Products from ALA are eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3).1,2

Precursors from both families compete as substrates for the same elongases (Elov-2 and Elov-2) and desaturases (Δ-5 and Δ-6, also called FADS1 and FADS 2, respectively) for the synthesis of LC-PUFAs.1,2 However, desaturases have higher enzyme affinity for omega 3 FAs, followed in preference by omega 6. When both FAs are deficient (ALA and LA), oleic acid is used, which is a precursor of the omega 9 family.1,2

EFA deficiency (ALA and LA) leads to a syndrome characterized by dry skin, growth retardation, hair loss, coagulation disorders, desquamation and hyperkeratotic dermatoses.1,3 Although neonates have the ability to elon-
gate and desaturate precursors of both families of FAs at early ages (from 28 weeks of gestation), this conversion to LC-PUFA is very limited. Biotransformation of ALA to DHA has been quantified from 0.04% to 5% in term and preterm neonates,\textsuperscript{4,5} which indicates that the tissue and plasma concentrations of these LC-PUFAs depend, in large measure, on the exogenous supply. Because of this, AA, EPA and DHA have been considered as EFAs because its production may be inadequate, such as in preterm neonates and during periods of rapid growth.\textsuperscript{3}

In addition, a study that evaluated the use of fish oil as the only source of lipids (with insignificant quantities of LA and ALA, but with a high content of EPA and DHA) in children with cholestasis associated with prolonged parenteral nutrition and fasting, no clinical or biochemical data regarding deficiency of EFA, hypertriglyceridemia or growth retardation were found. This reinforces the fact that these could be considered as EFAs and suggests that they may reduce the needs for ALA.\textsuperscript{3}

**Tissue Stores of AA and DHA in the Fetus and Newborn**

During pregnancy, the fetus receives a continuous supply of all nutrients through the placenta (including LC-PUFAs), so its supply depends on diet, adipose tissue reserves and maternal synthesis. As with other elements, accumulation or accretion of these exists during gestation. It was recently estimated in Western populations that fetuses have a tissue accretion of LC-PUFAs in the following order: LA>AA>DHA, and that the period of greatest accretion corresponds to the last 5 weeks of pregnancy. For LA, the average accretion is 342 mg/day, for AA 95 mg daily and for DHA 42 mg/day. Although AA accumulates in larger amounts in the fetus than DHA, at 40 weeks ~26% of DHA is found in cerebral tissue, whereas AA occupies only 11%.\textsuperscript{6}

DHA is the predominant FA in phospholipids of neuronal membranes in the cerebral cortex and the retinal photoreceptors. Its accretion during fetal life occurs during the period of genesis and neuronal differentiation, from about the sixth month of pregnancy, as well as the development of intense synaptogenesis and myelination in the early postnatal period, which continues during the first 2 years of life. AA and DHA increase almost 30 times in the frontal cortex of the human brain during fetal life and during the first 6 postnatal months. Therefore, DHA is associated with brain structures with a high level of importance in the development of cognitive and visual functions.\textsuperscript{7-10}

**Functions of AA and DHA**

AA and DHA are involved in many of the functional properties of cell membranes such as permeability, flexibility, transport properties, receptor activity, uptake and release of substances, signal transduction and conduction, as well as calcium and sodium ion flow. LC-PUFAs also have an effect on the expression of transcription factors for some genes such as peroxisome proliferator-activated receptor (PPAR α, γ) or in the translocation of nuclear factor κB (NF-κB) to activate genes of inflammatory molecules such as cytokines. Another mechanism by which DHA and EPA are involved in the regulation of inflammatory mediators is that they act as substrates for eicosanoid synthesis series 3 and 5, which are generally attributed to lower biological potency to induce vasoconstriction, bronchoconstriction, platelet chemotaxis and pain than those derived from AA series 2 and 4, respectively. DHA and EPA are also substrates for the synthesis of resolvins and protectins that modulate the magnitude of the inflammatory response and promote the process of inflammation resolution. Therefore, LC-PUFAs also have effects on the immune, inflammatory and allergic response locally and systemically in coagulation as well as in vascular and bronchial reactivity.\textsuperscript{11-15} A direct association has also been observed between AA content in erythrocytes and increase in weight and length of preterm infants.\textsuperscript{16}

**Effects of Supplemental AA and DHA Administration**

Supplementation of LC-PUFAs in formulas was derived from the observation that preterm infants fed with formula and who did not receive an exogenous DHA supply did not reach DHA blood levels in the same manner as breastfed infants, regardless of the content of its precursor (ALA) in the formula. Similarly, autopsy studies showed that the accumulation of DHA in brain, but not of AA, was higher in breastfed infants than those fed formula without DHA supplementation.\textsuperscript{8,17-19} For this reason, it is clear that DHA levels in blood and tissue of newborns depend on the exogenous supply such as mother’s milk in order to meet their requirement and, more so, in preterm newborns who did not complete their accretion during the third trimester.\textsuperscript{20}
**DHA and AA in Neurodevelopment and Visual Acuity**

Many studies have evaluated the effects of LC-PUFA supplementation on neurodevelopment and visual function. Although in preterm infants it has been found and confirmed that it offers benefits in both areas, for term infants there is no consistency according to the results. The difficulties in assessing the effects of supplemented DHA in infants may be influenced by differences in the accretion of DHA according to the weeks of gestation, period of lactation, maternal dietary intake and amount and ratio of AA and DHA administered in formulas, as well as the methodology for evaluation. For example, visual acuity or electrophysiological (electroretinogram) tests are more sensitive than clinical tests, which are based on the child's perception of different figures.  

The Cochrane Collaboration group performed a meta-analysis to evaluate whether administration of DHA and AA conferred advantages in regard to cognitive and visual development in term infants but did not find sufficient evidence of the usefulness of DHA-supplemented infant formula. In response to this publication, a group of researchers led by Dr. Uauy, one of the authors of the studies analyzed in the meta-analysis, indicated that there were differences in the supply of DHA to patients in the studies analyzed because formulas with different concentrations of DHA were used (0.1% to 0.36% of total FAs), i.e., there were studies whose interventions were three times lower than others as well as differences in the study design. Therefore, Uauy and Dangour reported that the conclusion of the Cochrane group that there was no benefit with LC-PUFA supplementation was invalid.  

The variability in the supply has been better controlled in other more recent studies where positive correlations have been found between DHA blood levels and improvement in visual and cognitive function among breastfed infants and infants fed with DHA-supplemented formula. The conclusion is that clinical trials that used formulas with DHA at doses ≥0.3% were more likely to have beneficial functional effects attributable to DHA.  

**DHA and AA on Growth**

The growth effect of LC-PUFAs administered with formula has shown inconsistent results. Whereas the high content of AA in tissue reserves has a positive effect on weight and length, initial studies in the 1990s suggested that DHA and EPA supplementation in formula diminishes growth.  

It was later reported that the negative effect of LC-PUFA n-3 on the growth of preterm and term infants was under certain experimental conditions and that this effect appears to have minimal clinical or physiological relevance. More recent studies where AA was also administered showed better results in weight and height gains without increasing morbidity or without adverse effects in stable preterm newborns.  

There are few studies that evaluated the effect of DHA as a sole intervention in critically ill neonates. In a study performed by our research group in neonates who developed clinical sepsis during their hospitalization, it was noted that on the 14th day of administration of 100 mg of DHA or olive oil each day, the DHA group showed an increase in weight (50 g, \( p = 0.03 \)) and on body fat (70 g, \( p = 0.03 \)) compared to the initial weight, whereas fat-free mass remained unchanged. Increase in height tended to be greater in the DHA group compared with the group that received olive oil (25 mm vs. 10 mm, \( p = 0.07 \)). The control group showed no gain in any of the variables evaluated, which indicated greater growth in the group that received DHA secondary to the decrease in the deleterious effects of sepsis. The latter was attributed to the early incorporation of DHA to the leukocyte membranes where it is probable that they were used as a substrate for the synthesis of mediators of inflammation. This also has a catabolic effect as was shown with the attenuation of circulating IL-1β. DHA supplementation also had a positive effect when was incorporated into leukocytes, which was related to a decrease in illness severity during hospitalization.  

**DHA and Retinopathy of Prematurity (ROP)**

Because the preterm infant had no sufficient time for DHA accretion in the retina, the newborn has disadvantages in the response to abnormal stimuli of vascular growth factors during postnatal life. During intrauterine development the fetal eye is found to be in a “hypoxic” environment, which stimulates vascular endothelial growth factor (VEGF) and the development of the factor similar to insulin (ILF-1) for the formation of vessels in the retina reaching 39 to 40 weeks, when complete retinal vascular development is achieved. In preterm infants, generally with <28 weeks of gestation, this development is halted by the greater level of environmental oxygen used for its treatment. This causes an avascular retina with hypoxia in some zones which, on increasing the receptor activity in retinal cells, increases...
the production of the mediators of vascular development (VEGF and IGF-1) in an abnormal and even excessive manner. In turn, this generates an alteration in retinal development referred to as retinopathy of prematurity (ROP) and that can lead to blindness. The mechanism by which DHA decreases retinopathy has been recently described in animal models. It is known that within the metabolism of prostaglandins, the lipooxygenase 5 enzyme adds a hydroxy radical to DHA and forms 4-hydroxy-DHA, which has an anti-angiogenic effect and is found in greater concentration where there are hyperoxic states. In rats that are genetically deficient in this enzyme, DHA administration showed no benefit on the decrease of angiogenesis in contrast to rats that did not have this enzymatic deficiency and did reduce retinal angiogenesis.31

An experimental study concerning neonates who had LC-PUFA n-3 supplementation from fish oil given along with the total parenteral nutrition demonstrated a decrease in the frequency and severity of ROP, which suggested that if the intrauterine content of DHA is insufficient, early and sufficient administration of DHA in newborns at risk may decrease the risk of developing ROP.32 This may be explained by the participation of tumor necrosis factor alpha (TNF-α) in the increase of angiogenesis because in DHA-supplemented newborns there was an increase in the production of resolvin D1 and neuroprotectin D1, 90% suppression of the TNF-α mRNA and also 30% suppression of the TNF-α protein. Therefore, it was concluded that the LC-PUFA n-3 could offer these infants protection against proliferative ROP due to the reduction of TNF-α.33

DHA, AA and Other Morbidities

There are studies that correlate the supply of DHA with decrease in the incidence of bronchopulmonary dysplasia (BPD) in preterm infants with birth weight <1250 g34,35 or of necrotizing enterocolitis (NEC) in newborns with weight between 725 and 1375 g.36 However, the benefit of DHA is not clear because these complications have a multifactorial origin37 and further studies are required to determine the role of DHA in these pathologies.

Recommendations for Supply of DHA and AA

Breast milk continues to be the first choice for neonatal feeding during the first 6 months of life.38 DHA concentrations in human milk may vary from 0.06% to 1.4% of the total FAs and from 0.24% to 1.0% of AA from the total FAs in women with babies born at term. In Mexican women, a content of between 0.42%39 and 0.43%40 from AA and between 0.17%40 to 0.26%39 of DHA has been reported. As was mentioned previously, the concentration of these FAs in maternal milk is related to its dietary intake, reserves in adipose tissue and with maternal synthesis, which explains the variability of the concentrations in maternal milk. Therefore, needs of the newborns are not necessarily fulfilled. In this context, it is recommended that during gestation and lactation, women include a daily consumption of 200–300 mg/day of DHA.7,38,40 To reach these doses, it is suggested that foods with a high content of DHA should be included in the diet of the mother, such as Atlantic cold-water fatty fish twice weekly at ~200 g portions (herring, salmon, halibut, cod, catfish)15 or species from the Gulf of México (red snapper, rubia, hake, yellowfin tuna, angelfish, and haddock).41 DHA can also be obtained through nutritional supplements especially for pregnant or nursing mothers.38 Mothers should be informed to avoid consumption of predatory fish because they are a source of contaminants such as dioxins, polychlorinated biphenyls (PCBs), heavy metals such as methylmercury, and others. The latter is particularly toxic in early development of brain and negatively affects the child’s growth. Among the most important species with the greatest content of methylmercury are shark, swordfish, marlin, king mackerel and pike.32,43 In the case of infant formulas, there are recommendations established to regulate their content (Table 1). These recommendations are based on strong evidence for the addition of DHA and AA in infant formula in the first 6 months life and have as a basis the average concentration of LC-PUFA n-3 in human milk, studies of visual acuity and cognitive development.36,44-50 In this regard, it is necessary to point out that even when the recommendations to supplement these formulas have been done with expert consensus, their quantities of DHA for term infants (minimum 0.2%–maximum 0.5% of total FAs) have been considered to be low6,51 because, as previously noted, when formulas with <0.3% of DHA and, AA with a ratio AA:DHA >1:1 was used, there was an association with improvement in cognitive results.4 Therefore, it has been proposed to take into consideration the high physiological DHA content of milk in women with regular fish consumption throughout their lives because it has been estimated that if a woman consumes 200–300 mg of DHA/day, this translates into a concentration of between 0.52
Docosahexaenoic acid and arachidonic acid in neonates: are they receiving a sufficient amount to meet their needs?

and 0.79% DHA of the total FAs in the milk, an amount above the actual recommendations. In the case of preterm infants, because DHA accretion during the third trimester was not completed, it is very probable that they will have a significant postnatal deficit that will increase with lower gestational age and weight. For this reason, quantities of DHA (0.35%) actually contained in supplemented formulas for preterm infants have been compared with formulas that supply 1% of DHA of total FAs, but the same quantity of AA. The authors have reported that cognitive development and visual acuity are still below the qualifications when they receive supplemented formulas with 0.35% of DHA compared with those reached by breastfed infants.

For this reason, the authors have suggested that a supply between 1 and 1.5% of the total lipids be provided to compensate for the early DHA deficit in preterm infants with birth weight <1250 g and enterally fed (which has been estimated to be 20 mg/kg/day and represents 44% of the DHA that should be accumulated in tissues). An amount of 1.5% of the total lipids corresponds to 111 mg of DHA/kg of weight per day if a feeding of 180 mL/kg/day of breast milk is taken into consideration. Similarly, breastfed infants 1-6 months of age who receive ~765 ml/day of maternal milk that contains an average of 41.3 g/l of lipids and who receive maternal milk with a high DHA content (1% of total lipids) are consuming ~315 mg DHA/day physiologically without demonstrating any adverse effects.

**Contents of Macronutrients and LC-PUFA in Commercial Formulas**

In Mexico there are formulas supplemented with DHA and AA available for term and preterm infants. The former formula contains between 8.6 and 17 mg DHA/100 kcal and between 8.6 and 34 mg of AA/100 kcal (Table 2), whereas milk formulas for preterm children contain between 10.8 and 25 mg of DHA/100 kcal and between 16.9 and 34 mg of AA/100 kcal (Table 3). According to the information reported in monographs, printed notes or manufacturers’ web pages, most laboratories offer formula options supple-
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Per 100 kcal</th>
<th>Energy, kcal</th>
<th>Proteins, g</th>
<th>Carbohydrates, g</th>
<th>Lipids, g</th>
<th>LA, mg</th>
<th>ALA, mg</th>
<th>Adequacy of AA at the minimum recommended %</th>
<th>DHA, mg</th>
<th>Adequacy of DHA at the minimum recommended %</th>
<th>Ratio of AA/DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mead Johnson</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>10.8</td>
<td>5.5</td>
<td>900</td>
<td>94</td>
<td>34.0</td>
<td>0.62</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
<tr>
<td>Nestlé</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>11.2</td>
<td>5.3</td>
<td>750</td>
<td>89</td>
<td>11.6</td>
<td>0.22</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
<tr>
<td>Wyeth</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>11.1</td>
<td>5.3</td>
<td>750</td>
<td>95</td>
<td>8.6</td>
<td>0.16</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
<tr>
<td>Nutricia</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>10.8</td>
<td>5.4</td>
<td>750</td>
<td>95</td>
<td>11.0</td>
<td>0.33</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
<tr>
<td>PBM Nutritionals</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>10.8</td>
<td>5.4</td>
<td>750</td>
<td>95</td>
<td>11.0</td>
<td>0.33</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
<tr>
<td>PISA</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>2.1</td>
<td>10.8</td>
<td>5.4</td>
<td>750</td>
<td>95</td>
<td>11.0</td>
<td>0.33</td>
<td>0.31</td>
<td>1.1</td>
<td>2:1</td>
</tr>
</tbody>
</table>

LA, linoleic acid (n-6); ALA, α-linolenic acid (n-3); AA, arachidonic acid; DHA, docosahexaenoic acid; NA, not applicable; —, not indicated by the manufacturer.
Docosahexaenoic acid and arachidonic acid in neonates: are they receiving a sufficient amount to meet their needs?

Table 3. Nutritional composition and n-3 and n-6 FA in formulas for preterm babies according to the manufacturer

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Mead Johnson</th>
<th>Nestlé</th>
<th>Wyeth</th>
<th>Abbott</th>
<th>PISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition/100 kcal</td>
<td>Enfamil Premature Powder</td>
<td>Pre-NAN Powder</td>
<td>Good Start Supreme Premature Liquid</td>
<td>Good Start Supreme Premature Powder</td>
<td>SMA Premature Gold Powder</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Energy, kcal/oz.</td>
<td>24</td>
<td>27</td>
<td>24</td>
<td>24</td>
<td>24.8</td>
</tr>
<tr>
<td>Proteins, g</td>
<td>3</td>
<td>2.8</td>
<td>3.0</td>
<td>2.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>11</td>
<td>10.6</td>
<td>10.4</td>
<td>10.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Lipids, g</td>
<td>5.1</td>
<td>5.2</td>
<td>5.2</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Medium-chain triacylglycerols, %</td>
<td>40</td>
<td>30.7</td>
<td>40</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>LA, mg</td>
<td>810</td>
<td>810</td>
<td>1000</td>
<td>790</td>
<td>732</td>
</tr>
<tr>
<td>ALA, mg</td>
<td>110</td>
<td>97</td>
<td>98.8</td>
<td>102.6</td>
<td>—</td>
</tr>
<tr>
<td>AA, mg</td>
<td>34</td>
<td>19.5</td>
<td>32</td>
<td>19.7</td>
<td>31</td>
</tr>
<tr>
<td>DHA, mg</td>
<td>17</td>
<td>19.5</td>
<td>16</td>
<td>19.7</td>
<td>21</td>
</tr>
<tr>
<td>AA, %</td>
<td>0.67</td>
<td>0.38</td>
<td>0.62</td>
<td>0.38</td>
<td>0.57</td>
</tr>
<tr>
<td>Adequacy of AA at the maximum recommendation, %</td>
<td>111</td>
<td>63</td>
<td>103</td>
<td>63</td>
<td>95</td>
</tr>
<tr>
<td>DHA, %</td>
<td>0.33</td>
<td>0.38</td>
<td>0.31</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>Adequacy of DHA at the maximum recommendation, %</td>
<td>94</td>
<td>109</td>
<td>89</td>
<td>109</td>
<td>111</td>
</tr>
<tr>
<td>AA/DHA ratio</td>
<td>2:1</td>
<td>1:1</td>
<td>2:1</td>
<td>1:1</td>
<td>1.5:1</td>
</tr>
</tbody>
</table>

FA, fatty acids; LA, linoleic acid; ALA, alpha linolenic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; —, not indicated by the manufacturer.

Veterans with LC-PUFA (Tables 2 and 3). If we take into consideration the minimal content that the formulas should contain according to the international recommendations of 0.2% for DHA and AA based on ESPGHAN, Codex Alimentarius and the U.S. Steering Committee, these formulas for preterm infants (0-6 months) have a percentage of adequacy of between 80 and 155% for DHA and between 95 and 315% for AA. However, according to studies with positive effects on neurodevelopment and visual acuity, which demonstrated that the recommended minimum of total FAs is 0.3% for DHA and AA, these formulas would have a percentage of adequacy of between 53 and 103% for DHA and between 53 and 210% for AA for the suggested requirement for term infants.

Considering formulas for preterm infants, the minimum suggested values are not clear; therefore, if the maximum established recommendations by consensus in 2002 are used (0.35% for DHA and 0.6% for AA), these formulas supply between 57 and 131% of DHA and between 53 and 112% for AA. However, if we take into consideration the suggested percentage of 1.5% of DHA to compensate for its accretion deficit, the formulas for preterm infants barely cover between 13 and 31%. Despite this, it is noteworthy that the suggested AA:DHA ratio of at least 1:1 was observed in all supplemented formulas.

In conclusion, we can state that, depending on the conditions of the child’s adaptations according to their gestational age, their tissue reserves of AA and DHA, with added inflammatory disease (which increases the use of DHA and AA as substrates for mediators of inflammation) and DHA and AA intake with breast milk, the needs of each patient vary and may be higher than current recommendations. In this regard, the child may present deficiencies that are not necessarily manifested clinically.
For these reasons, it is recommended that neonates receive breast milk as the first feeding option along with the advice for mothers to eat foods high in DHA (fish) or use supplements to achieve between 200 and 300 mg of DHA per day. If there is no breast milk available, using formulas supplemented with DHA and AA is beneficial to the growth and development of infants, especially for those preterm, even if the DHA content of some formulas does not fulfill the specific needs of the newborn.

REFERENCES

7. Guesnet P, Alessandri JM. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)—Implications for dietary recommendations. Biochimie 2011;93:7-12.
30. López-Alarcón M, Bernabe-García M, Del Valle O, González-Moreno M, Villegas R. Oral administration of docosahexaenoic acid attenuates interleukin-1β response and clinical course of...
42. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 2006;296:1885-1899.
Coping strategies and their relation with depression and anxiety in pediatric residents in a third-level pediatric hospital

Ana Carolina Sepúlveda-Vildósola,1 Ana Laura Romero-Guerra,2 Leonel Jaramillo-Villanueva3

ABSTRACT

Background. Depression and anxiety are common among medical residents. Coping strategies are cognitive and behavioral efforts in order to manage stress and specific individual demands. The use of active strategies has been associated with a lower frequency of anxiety and depression. Our objective was to determine if there is an association between depression and anxiety and the coping strategies used by medical residents.

Methods. Previous consent, Beck Anxiety and Depression Inventory and Coping Strategies Inventory were completed. Data were analyzed using χ² test.

Results. A total of 112 residents were included. Depressive symptoms were identified in 39.6% and anxiety in 25.6%, with coexistence of symptoms in 21.4% of subjects. Anxiety was associated with the type and year of residency. Two of the passive subscales of coping strategies were associated with depression and three were associated with anxiety.

Conclusions. Depressive and anxiety symptoms are frequent among medical residents. Mixed coping strategies are most commonly used. Passive coping strategies are associated with depression and anxiety.

Key words: depression, anxiety, medical residents, coping strategies.

INTRODUCTION

Mental disorders have a strong impact on the lives of individuals, families and society. It is estimated that >20% of the world population will suffer from an affective disorder that will require medical treatment at some point in their lives1 and that by the year 2020 depression will be the second leading cause of years of healthy life lost worldwide.2 In Mexico, neuropsychiatric disorders occupy fifth place as burden of disease and is an indicator of premature death and days lived with a disability.3 The National Psychiatric Epidemiology Survey (2002) found that the prevalence of depression in adults in Mexico was 4.5% and that affective disorders are ranked in third place according to their frequency.4

Service professions involved with lives, among which medicine is one, have been linked to the increased prevalence of these diseases due to the number of professionals coupled with the stressors of daily life. As these are experienced, resolved and addressed, they determine an individual’s response to stress. This may result in adaptive or maladaptive behaviors causing wear and personal dissatisfaction and are reflected in professional and mental health.

Physicians in specialty training are also subject to other stressors related to this formative period, such as long work hours, sleep deprivation, academic and employment requirements, conflicts with other staff members, sense of insecurity for future employment and situations such as the death of patients, conflicts with family or friends, financial difficulties or moving to a new city, among others, which makes them even more susceptible to psychiatric disorders,
particularly depression and anxiety. Other conditions described in this group are addiction and somatization, as well as family conflict. In 2010, researchers at the University of California found that up to 60% of medical residents met the DSMIV criteria for major depression. In 2005, at the Hospital de Pediatria (Pediatric Hospital), Centro Medico Nacional Siglo XXI, a prevalence of 64% was found for depression and 47% for anxiety among pediatric residents, numbers far greater than those of the general population. Another study carried out in Canada among family medicine residents described a lower prevalence of depression and anxiety (20 and 12%, respectively), although this represented three to four times more than the general population of that country.

Stress is a phenomenon resulting from the relationship between the person and the events of their environment, which are assessed as disproportionate or threatening according to their resources and may endanger their welfare. The individual must implement strategies that allow them to manage the stress. Coping is a cognitive effort and a behavioral dynamic, whose management is oriented to reduce, minimize, master or tolerate these external and internal demands that generate stressors. Coping is an important aspect of personal functioning and is divided into styles and coping strategies. According to Pelechano, these concepts are complementary. The first ones are personal predispositions to address various situations. They are stable, temporary and situational and consistent and determine the use of certain strategies. Strategies are concrete and specific processes used in each context and may be changing, depending on the condition triggers. Strategy has advantages such as its modifiability and greater predictive power, becoming more attractive from the perspective of the intervention. Personal factors such as stable couple relationships and the presence of children have been associated with improved implementation of coping strategies. Coping strategies are classified into primary and secondary scales or subscales. The primary strategies are as follows:

- **Active**—behaviors related to the problem, analyzing the circumstances to determine the course of action, reflection of possible solutions, search for information, strategies for anticipation for a disaster, and control of emotions and circumstances as well as seeking social support.

- **Passive**—use of strategies such as withdrawal; not addressing the problem; not experiencing any emotions; ignore, deny or dismiss the event; joking and taking things lightly and passive acceptance.

Active or adaptive strategies have a neurobiological position in the areas of logical-formal thinking (frontal areas), whereas passive strategies are dependent on instinctual mechanisms (limbic system).

In turn, these strategies are divided into eight subscales or secondary strategies (Table 1). It has been reported that avoidance predicts stress and depression in the future. This coping strategy is seen with greater frequency in subjects less familiar with the appropriate management of adversity. Some studies report that individuals with anxiety disorders experience predominantly emotion-focused approach and avoidance-escape (passive strategies), whereas individuals without psychiatric disorders obtained higher scores on the dimensions focused on the problem and evolution (active strategies). In studies conducted in other countries with medical residents, results found that these residents tend to use inappropriate strategies in up to 32.7%, mainly those who were younger or who were in the first year of the specialty.

There are few instruments to assess coping strategies. The coping strategies inventory (CSI) is validated and reliable (Cronbach’s alpha 0.76–0.86), which includes qualitative and quantitative information on the frequency of the use of certain coping strategies. The instrument consists of 40 questions, five for each secondary strategy.

The Beck Depression Inventory (BDI) is used worldwide to quantify the presence and severity of depressive symptoms. It was adapted and validated in Spanish in 1975 (coefficient alpha 0.81–0.86). Finally, the Beck Anxiety Inventory assesses the severity of symptoms of anxiety and differentiates from the depressive symptoms. It can be used in the psychiatric as well as normal population. The reliability index for the total scale is 0.83.

At the Hospital de Pediatria (Pediatric Hospital) of the Centro Medico Nacional Siglo XXI, there has been an increase in depression and anxiety attacks in some pediatric residents and medical specialties during the last 3 years. We wanted to determine the coping strategies used by the residents as well as the frequency of depression and anxiety symptoms. We also wanted to establish a possible relationship between these coping strategies and frequency of depressive and anxiety symptoms.
SUBJECTS AND METHODS

After approval by the Local Research and Ethics Committee, all residents assigned to the hospital during the month of December 2011 were invited to participate. We excluded those who were on vacation or were working in external rotations. A survey of sociodemographic data was collected. Residents were asked if at the time of the survey they were on any medication for depression or anxiety. If affirmative, they were excluded from the study. The remainder of the residents proceeded to answer the questions for Coping Strategies, Depression and Beck Anxiety. We eliminated those subjects who did not complete the survey. Residents used pseudonyms.

The Beck Depression Inventory was scored according to a scale of 0–63 points, with each answer worth 0 to 3 points. The total sum was obtained and interpreted as follows: ≤9 without depressive symptoms; 10–16 mild symptoms; 17–29 moderate; and ≥30 points as severe depressive symptoms. The Beck Anxiety Inventory was scored on a scale of 0–63 points, each answer worth 0–3 points. Those with ≤15 points were without anxiety symptoms; 16–21 points indicated had mild symptoms; 22–35 points were moderate symptoms, and ≥35 points represented severe anxiety.

The Coping Strategies Inventory was evaluated as follows: for each response "in absolute" was given a score of 0; for "little" a score of 1; for "enough" a score of 2; for "too much" a score of 3; and for "total" a score of 4. The present strategy was considered when three responses were reported with a score from 2–4 (from "enough" to "total") for the five questions, and it was felt that presenting 3/4 subscale areas was present predominantly active or correspondingly passive. Mixed strategies were considered in those who presented with more than two subscales of each primary strategy simultaneously. Those who submitted responses between 0 and 1 in the different subscales were considered as "none."

Descriptive analysis of the qualitative data is expressed as a percentage, and quantitative, average and standard deviation. Inferential statistical analysis was performed using χ² test; p ≤0.05 was considered statistically significant. Results were published under the pseudonyms of those subjects who were identified with depression or anxiety, and those who applied were referred to a mental health service of the hospital.

RESULTS

From a total of 202 residents assigned to the hospital (including pediatric residents in their first year who are in their secondary rotation); 90 residents were excluded for the following reasons: nine for being under psychiatric treatment; 12 for being in a field rotation; 18 for being on vacation or working in external rotations; 16 who failed to answer the survey and 34 who did not attend the meeting for implementation of the instrument. One subject was excluded due to an incomplete questionnaire.

Eventually, 112 participants (55.44%) were included. Their demographic characteristics are detailed in Table 2; 53.6% were from pediatric specialties and the remainder from other specialty areas. Twenty residents (17.8%) were
Coping strategies and their relation with depression and anxiety in pediatric residents in a third-level pediatric hospital

For anxiety, symptoms were reported with a frequency of 24.1% \((n = 27)\), 33.3% \((n = 9)\) having mild symptoms, 59.3% \((n = 16)\) with moderate anxiety symptoms and 7.4% \((n = 2)\) with severe anxiety symptoms. Coexistence of symptomatology was identified in 22.3% \((n = 25)\) of the subjects. In a global analysis of the included subjects and those excluded for presenting previous psychiatric pathology, the prevalence of anxiety symptoms was 25.6%, depressive symptoms 39.6% and coexistence of symptoms 21.4%.

No association was found with the presence of severity of depressive symptoms between gender \((p = 0.65)\), age \((p = 0.46)\), years of residency completed \((p = 0.258)\), chosen specialty \((p = 0.412)\), marital status \((p = 0.157)\), presence of children \((p = 0.622)\) or rotation service \((p = 0.66)\). Despite not finding a statistically significant association, 55% of first-year residents, 53.3% of second-year residents and 41.6% of fourth-year residents presented depressive symptoms compared with 27.7% in third-year residents, 24% in fifth-year residents and 33% of sixth-year residents.

### Table 2. Demographic characteristics of the group of pediatric residents and second branch of specialty

<table>
<thead>
<tr>
<th>Excluded due to previous psychiatric pathology</th>
<th>Included</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 9)</td>
<td>%</td>
<td>(n = 112)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td><strong>Year of residency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Second</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Third</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fourth</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td>Fifth</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>Sixth</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td>Seventh or higher</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Civil status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>Married</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td>Free union</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td><strong>Age (years) (median ± SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.67 ± 1.225</td>
<td>min 29, max 33</td>
</tr>
</tbody>
</table>

NS, not significant; SD, standard deviation.

enrolled in their first year (pediatrics and genetics), 15 (13.3%) were in their second year (pediatrics and genetics), 18 (16%) were in their third year (pediatrics, pediatric surgery and genetics), 12 (10.7%) were in their fourth year (pediatrics and pediatric surgery), 25 (22.3%) were in their fifth year (second specialty and pediatric surgery), 21 (18.7%) were in their sixth year (second specialty and pediatric surgery) and one in their seventh year (pediatric neurosurgery). The highest percentage of included subjects corresponded to third-year residents (66%), and the group with the highest percentage of loss was fourth-year residents (49%) because they were physically outside the unit in a field rotation.

Four of the nine students who were excluded due to pre-existing disease were in pediatric specialties (44.4%), and the remainder represented other specialties. We identified a frequency of depressive symptoms in 37.5% \((n = 42)\) of the subjects studied; 73.8% showed mild depressive symptoms \((n = 31)\) and 26.1% \((n = 11)\) demonstrated moderate symptoms. No subject was identified with severe depressive symptoms.
With regard to anxiety symptoms, there also was no association with gender \( (p = 0.10) \), age \( (p = 0.515) \), specialty \( (p = 0.287) \), marital status \( (p = 0.687) \), number of children \( (p = 0.778) \) or the rotation service \( (p = 0.70) \). However, there was an association between the presence of anxiety symptoms and the year of residency \( (p = 0.012) \), finding that 35% and 40% of the residents in their first and second year, respectively, had anxiety. Regarding the severity of anxiety symptoms, it was observed that for first-, second- and third-year residents there were predominantly moderate and severe symptoms.

When analyzing symptoms according to the type of specialty, it was found that 33% of the pediatric residents presented depressive symptoms compared with 13.4% of residents representing other specialties \( (p = 0.012) \). The same was true for anxiety because it was found that 45% of the pediatric residents displayed symptoms of this spectrum, whereas only 28.8% of residents from other specialties presented this \( (p = 0.058) \). No statistically significant difference was found, but it was clear that almost twice the number of pediatric residents had anxiety symptoms.

Analysis of the percentages of students who presented with primary coping strategies is detailed in Table 3. Contrary to expectations, no significant association was found between primary coping strategies (active vs. passive) with the frequency of depressive symptoms. However, qualitatively, we found that 25% of those using active strategies presented depressive symptoms unlike 40% of those using predominantly passive strategies or 44% of those using mixed strategies, as was expected.

Regarding anxiety symptoms, only 7% of the students using active strategies had anxiety symptoms, whereas 40% of those using predominantly passive strategies and 31.9% of those using mixed strategies had some anxiety symptoms.

Coping strategies were not related to other sociodemographic variables or related to the resident’s specialty [gender \( (p = 0.72) \), year \( (p = 0.630) \), marital status \( (p = 0.250) \) or the presence or absence of children \( (p = 0.486) \)].

Depressive symptoms were not associated with either primary coping strategies such as active/adaptive or with secondary strategies (problem solving \( p = 1.0 \), cognitive restructuring \( p = 0.364 \), social support \( p = 0.532 \), or emotional expression \( p = 0.432 \)). Anxiety symptoms also had no association with those primary and secondary strategies \( (p = 0.448, p = 1.0, p = 1.0 \text{ and } p = 1.0, \text{ respectively}) \).

No association was found between depression and the prevalence of primary passive strategy or for two of their subscales, avoidance of problems \( (p = 0.517) \) and social withdrawal \( (p = 0.171) \); whereas wishful thinking and self-criticism are significantly associated \( (p = 0.005 \text{ and } p = 0.003, \text{ respectively}) \). No associations were found between anxiety and predominantly passive primary strategy or with the social withdrawal subscale \( (p = 0.80) \). However, we did find a significant association with other passive strategies (avoidance of problems \( p = 0.027 \); wishful thinking \( p = 0.011 \), and self-criticism \( p = 0.002 \)). The number of secondary strategies employed is also significantly associated with the presence of anxiety symptoms \( (p = 0.016) \). The use of three or more passive strategies was presented in 92.5% of the students with symptoms of anxiety (Table 4).

### DISCUSSION

In the present study we found that the frequency of depressive symptoms was 39.6%, a figure much higher than that found in 2005 by Bello et al. in the Mexican adult population (4.5%), but less than that found 7 years prior among residents of the same hospital (60%). These results could be explained by the use of different measurement instruments, and the measures taken to detect and treat early depressive symptoms among residents. Of all the residents with depressive symptoms, 75% corresponded to mild grade, and none presented with severe depression. Only nonpharmacological intervention was required. First- and second-year residents presented anxious and depressive symptoms in a greater proportion followed by fourth-year residents. These facts may be explained because first-year residents experience great uncertainty.
Coping strategies and their relation with depression and anxiety in pediatric residents in a third-level pediatric hospital

Table 4. Frequency of secondary coping strategies used by the medical residents

<table>
<thead>
<tr>
<th>Number of strategies used</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Active strategies</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problem resolution</td>
<td>92</td>
</tr>
<tr>
<td>Cognitive restructuring</td>
<td>88.4</td>
</tr>
<tr>
<td>Social support</td>
<td>90.2</td>
</tr>
<tr>
<td>Emotional expression</td>
<td>83.9</td>
</tr>
<tr>
<td>Avoidance of problems</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive strategies</td>
<td>%</td>
</tr>
<tr>
<td>Wishful thinking</td>
<td>85.7</td>
</tr>
<tr>
<td>Social withdrawal</td>
<td>55.4</td>
</tr>
<tr>
<td>Self-criticism</td>
<td>68.8</td>
</tr>
</tbody>
</table>

about their profession because they are novices during this formative period while having to assume diagnostic and therapeutic responsibilities of patients. Second-year residents may experience anxiety and depressive symptoms due to their being a lower-level resident according to the hospital pyramid along with a heavy academic load, rotations in intensive care units, more frequent rotations and being under the constant supervision of other medical staff members. Finally, fourth-year residents who are nearing graduation in their specialty may experience the uncertainty of their academic and employment future, in addition to the pressure of certification exams, graduation and job selection. Pediatric residents have more depressive symptoms than residents working in other specialties, probably because they are younger, unmarried and this may be their first experience away from home and families. Contrary to what was reported in the international literature, we did not find any association between female gender and the presence of depressive symptoms.

Anxiety is an emotional response that occurs when one perceives or interprets situations as threatening or dangerous, and its effects produce physical and emotional wear. The anxious behavior affects many aspects of a person’s daily life such as efficiency in problem-solving, interactions with other persons, the manner in which they describe themselves, their ability to relax, their quality of life and, thus, their health. In our study population these alterations may favor the presence of errors in the management and evaluation of patients while impacting negatively on the physician–patient relationship. There are few studies describing the prevalence of these diseases. In 2005, Virgin-Montelongo et al. reported that 14.8% of the Mexican population had some sort of anxiety disturbance and Malagon-Calderon and Gonzalez-Cabello reported a frequency of 47%. In this population we identified a frequency of anxiety symptoms in 25.6% of the subjects, which also means a reduction from what was reported 7 years ago. However, the highest proportion of residents was classified with moderate anxiety, and 22% of the residents experienced both disorders. These data should encourage us to find solutions to these problems because a third of our residents had moderate levels of anxiety and almost half of them had some degree of depression, which necessarily influences their academic achievement and job performance.

It is widely known that external stressors may be related with the presence of depression and anxiety. However, this study found no such association except for the type and degree of specialization. It was also noted that only 25% of the residents use active coping strategies, which would protect against the presence of symptoms of depression and anxiety. The working hypothesis was confirmed because a much lower percentage of residents presented anxious or depressive symptoms compared with those using predominantly passive or mixed strategies (depression occurred in 25% of the active coping vs. 40% and 44% for passive or mixed coping, respectively, and for anxiety and 7% vs. 40% and 31%, respectively). However, the low proportion of subjects using appropriate strategies should alert us to establish training programs in an attempt to modify strategies to confront stress. Approximately 70% of our residents use inappropriate strategies (passive and mixed), which is twice that reported by Pelechano. This author reported that the youngest and first-year residents were more likely to have inadequate coping strategies. In our population we found no association with any of
these variables, although there was a slight decrease in the percentage of students who use passive or mixed strategies when they were second specialty residents (70, 73, 72, 75, 64 and 62% for the first, second, third, fourth, fifth and sixth years, respectively).

Likewise, it was observed that 36% of the sample used one or more passive strategies, which implies that irrational responses prevailed. Of these, wishful thinking, defined as the forming of opinions and making decisions based on what would be most pleasing to imagine instead of substantiated evidence or rationality, reflected the wish that the reality was not stressful. This strategy was used by 85.7% of the subjects and was associated with elevated symptoms of anxiety and depression, which shows the difficulty in dealing with situations and, at the same time, represents a compensatory mechanism to decrease the discomfort created by the stressful conditions.

Avoidance of the problem, which refers to strategies including denial and avoidance of acts or thoughts related to the stressful event, also presented a frequency of 71.4% and was significantly associated with symptoms of anxiety, which is indicative of maladaptive emotional responses by the inability to adapt to stressors. It was noted that demographic variables involved in increasing anxiety symptoms were found in newer residents, so it follows that the most important stressors were lack of experience in pediatric patient care and confrontation of a place that has unknown work dynamics. Likewise, it was considered that this point is important because a strategy of avoidance motivated by situations of anxiety may be misinterpreted by general practitioners or teachers at any given time, such as a lack of commitment or collaboration of the resident with the patient care activities, which may generate work disputes.

On the other hand, self-criticism defined as self-incrimination, and self-criticism for the occurrence of the stressful situation and its mismanagement,1 were other passive strategies frequently used (68%) and that were associated with symptoms of anxiety and depression. Unlike the two strategies already reported, these are focused on emotion, denoting a coping strategy based on self-incrimination and less “up-front” behaviors, in other words, unsound for addressing the problems.

Finally, social withdrawal, defined as the exit strategy of friends, family, colleagues and significant others, is associated with the emotional reaction to stressful process, was presented in 55.4% of the population. This strategy was not associated with symptoms of depression and anxiety. However, it is important to note that this strategy reflects the lack of support networks and the risk involved: increase in emotional distress and the possibility of withdrawing from medical residency.

REFERENCES

Coping strategies and their relation with depression and anxiety in pediatric residents in a third-level pediatric hospital


Frequency of infection and disease due to cytomegalovirus and risk of development in pediatric kidney transplant patients

María Antonia Julián Núñez, María Guadalupe Miranda Novales, Eric Moisés Flores Ruiz, Ignacio Guerra Gallo, Fortino Solórzano Santos, José Guillermo Vázquez Rosales

ABSTRACT

Background. In regard to transplant-associated complications, infections represent one of the principal causes of death and graft loss. Of these, viral infections are the principal cause. The aim of this study was to determine the frequency of infection and/or disease due to cytomegalovirus (CMV) and clinical presentation in a cohort of pediatric kidney transplant patients as well as to present the risk factors associated with its development.

Methods. We carried out a retrospective cohort study. Clinical files of patients who underwent kidney transplantation between 2004 and 2006 and with a minimum of 6 months of follow-up were reviewed. Active infection was considered if a positive IgM to CMV after transplant was detected or seroconversion or positive pp65 antigenemia or CMV-DNA positive PCR test. Cases represented patients with active infection or disease and controls were patients without infection and/or disease. Risk factors investigated were age, serological status previous to transplant, donor, drug prophylaxis, year of transplant and blood transfusions.

Results. Of 120 transplant patients, 81 fulfilled the inclusion criteria; 53% were male and 47% female with a median age of 12 years. During follow-up, four patients presented a probable infection (4.9%), ten patients had active infection (12.3%), two patients had CMV disease (2.5%), and one patient presented rejection plus infection (1.2%). Fifteen patients presented rejection due to different causes (18%) and 49 patients (60.5%) did not develop complications. The only significant risk factor for development of infection or disease was a previous negative serological status for CMV (OR 3.58 95% CI 0.94-14.74, p = 0.035). Frequency of infection was higher in the year 2004 (12/17 patients). Prophylaxis was administered correctly in only 28.9% of the patients.

Conclusions. Frequency of CMV infection in pediatric kidney transplant patients was 20%. Among high-risk groups, frequency was 34% and decreased to 9% among low-risk groups. Most infected patients were asymptomatic, 30% presented general symptoms and 10% presented specific signs of CMV disease. Serological diagnosis was performed for most of the cases (IgM to CMV). The only significant risk factor for development of CMV infection was negative serological status for CMV prior to transplant.

Key words: cytomegalovirus, renal transplant, rejection of renal allograft.

INTRODUCTION

Renal transplantation can be considered the optimal replacement therapy for pediatric patients with end-stage renal disease (ESRD) and its objective is to restore physical and intellectual capacity and emotional development and to reduce mortality. With regard to complications associated with transplants, infections represent one of the principal causes of death and graft loss, occurring more frequently during the first year after transplantation. Of these, viral infections are the leading cause of morbidity and mortality with cytomegalovirus (CMV) being responsible for 70% or more of febrile episodes between the first and sixth months after transplantation, coinciding with the period of maximum immunosuppression.

In patients who are immunocompetent, the majority of primary infections as a result of CMV are asymptomatic, but in transplant patients, infection and disease presentation varies according to the type and nature of the transplanted organ, integrity of cellular and humoral response, type and duration of immunosuppressive therapy, and use of antilymphocyte preparations as well as the use of blood from donors positive for CMV. A review of
16 studies that included 1276 patients with solid organ transplants found that the percentage of CMV infection after transplantation was 70%; however, in renal transplant patients, this percentage increased to 84% in seropositive patients. 

Asymptomatic infection is viral replication in the absence of symptoms and CMV disease is defined as the presence of symptoms in association with the same viral replication. In transplant patients, CMV is not only present in a particular organ but is also generalized, which is known as CMV syndrome and defined as the presence of fever (>38°C) for at least 2 days during a period of 4 days, myalgia, neutropenia or thrombocytopenia, elevated transaminases and increased CMV antigen titers.

Invasive disease occurs in patients with severe immunocompromised state, causing spread of disease as well as pneumonitis, hepatitis or gastrointestinal disease.

Serological status to CMV of the donors (D) and recipients (R) prior to transplantation determines the incidence and severity of the disease. Thus, in seronegative individuals receiving latently infected cells of seropositive donors (D+/R-), CMV infection occurs primarily with systemic dissemination and 50-65% of patients manifest an acute disease. In seropositive individuals before transplantation with seropositive or seronegative donors (D+/R+ and D-/R+), the endogenous virus is reactivated when conventional immunosuppressive therapy is used and ~10-15% directly presented infection.

Because CMV infection is ubiquitous in the population and can be acquired early in life, its diagnosis is complicated and determination of the immune response alone is not sufficient. Culture isolation, PCR identification of viral nucleic acids, and identification of early antigens for CMV (pp65) using leukocyte fluorescence have improved diagnostic sensitivity. The latter two techniques are able to detect the virus even 2 weeks prior to the appearance of clinical symptoms and thus be suitable for early initiation of therapy in asymptomatic CMV infection.

Two treatment strategies have been introduced for transplanted patients: prophylactic treatment and anticipated treatment, with the objective of the former being prevention of CMV infection and symptomatic disease and for the latter only disease prevention, both decreasing the incidence of acute rejection, graft loss and death.

The prophylactic approach involves systemic administration of antiviral medications to all high-risk patients for CMV infection, initiated immediately after the transplant and with a duration of 21-100 days. In a review by the Cochrane Renal Group that included studies on the administration of acyclovir and gancyclovir compared to placebo, a 40% decrease was found in regard to the relative risk for development of CMV infection and 50% decrease for the development of the disease in patients who received antiviral treatment, with better results for gancyclovir. In a recent meta-analysis, a protective effect for disease (80% reduction) with the administration of antiviral prophylaxis was demonstrated. On the other hand, anticipated therapy is the administration of a specific drug when viremia is detected in a timely manner, prior to onset of clinical symptoms. Although its duration is variable, most authors administer the drug for 14-21 days or until resolution of the viremia. Its use is recommended on the basis of a lower probability of toxicity, developing resistance and lower costs because patient selection is based on those who are at high risk for developing the disease. Until now, a 72% effectiveness in disease prevention by CMV has been reported. However, a recent noncomparative study utilizing valgancyclovir in adult patients with solid organ transplants showed an efficacy of 100%. A gradual reduction has also been reported of the antigenemia level of CMV during the first weeks of anticipated therapy. Comparative studies in adult subjects with anticipated and prophylactic therapy demonstrated a moderate difference in risk reduction for developing disease. In general, the statistical arguments favor anticipated therapy instead of prophylactic therapy. However, it is necessary to use the sensible and dependable method to determine active infection in a timely manner and to establish an early treatment modality.

The objective of this study was to determine the frequency of infection and disease by CMV in a cohort of pediatric patients with renal transplant, to report their clinical characteristics and to determine the risk factors associated with CMV development.

PATIENTS AND METHODS

We carried out a retrospective cohort of patients with ESRD subjected to renal transplant during the years 2004-2006 in the Hospital de Pediatría (HP), Centro Medico Nacional Siglo XXI (CMNSXXI), Instituto Mexicano del Seguro Social (IMSS) in Mexico City, a tertiary care
hospital serving patients from the southern and southeast regions of Mexico. Patients 1-16 years of age were selected and who had a complete medical record and with at least 6 months follow-up after transplantation. Data collected corresponded to age, gender, cause of ESRD, type of donor (cadaveric or living related), serological status for CMV of the donor and recipient (D+/R+, D-/R+, D+/R-, D-/R-), immunosuppressive therapy used, pharmacological used for CMV prophylaxis that was graded according to the drug administered, dose, time of administration and donor type (Table 1). Moreover, treatment for symptomatic or asymptomatic CMV infection was collected, classifying this as adequate or inadequate: asymptomatic administration of valgancyclovir (15 mg/kg/dose) every 12 h for 21 days or gancyclovir i.v. (5 mg/kg/day) every 12 h for 21 days or symptomatic: gancyclovir (5 mg/kg i.v.) twice a day for 21 days.

Outcome variables were as follows:

a) suspected CMV infection defined as the probability of infection based on clinical or laboratory data without laboratory confirmation (pp65 antigenemia or PCR or serology)

b) active CMV infection, asymptomatic patient, positive for pp65 antigenemia, real-time PCR, seroconversion or presence of specific IgM

c) CMV disease with the presence of signs, symptoms and laboratory abnormalities suggestive of symptomatic infection that may be either one of two types: 
1) viral syndrome: fever >38°C without other obvious source of infection, PCR or antigenemia positive for CMV and one of the following findings: I) leucocytes ≤4000/mm³, II) atypical lymphocytes >3%, III) platelets ≤100,000/mm³, or 2) disease with tissue invasion with CMV infection focused on an organ or tissue demonstrated by histopathology

d) rejection of the transplanted organ due to CMV infection, presence of CMV in organ transplant detected by histology or PCR

e) rejection due to other causes such as rejection of the transplanted organ without identification of CMV infection

f) mortality associated with CMV with death within 6 weeks of diagnosis of CMV disease determined by molecular biology techniques, biopsy or autopsy

Analyzed risk factors for CMV infection or disease were age <11 years, high-risk recipient (D+/R-), cadaveric donor graft, red cell transfusion during transplantation, inadequate pharmacological prophylaxis recommendation against CMV (omitted prophylaxis, inferior or incomplete or insufficient or incomplete and insufficient) and transplant performed during 2004.

Statistical Analysis

Descriptive statistics were used to establish the prevalence of the phenomena observed in the population and χ² test was used for establishing differences in proportions of a predictive variable among patients who developed and did not develop infection or disease. Using bivariate analysis, odds ratios and 95% confidence intervals were calculated to evaluate the risk of developing infection or disease and then performing multivariate analysis using logistic regression in a saturated model.

Ethical Aspects

Because this was a chart review, it was classified as a minimal low-risk study according to the Health Law Statute (Article 17). The study was approved by the Local Research Committee. All information was obtained from records in a confidential manner.

RESULTS

Description of the Cohort

During the period from January 2004 to December 2006 there were 115 kidney transplants performed in the HP CMNSXXI: 39 in 2004, 41 in 2005 and 35 in 2006. Of these, 39 did not meet the inclusion criteria mainly due to lack of medical records, leaving 81 transplants corresponding to 80 patients (one patient was transplanted twice). Males comprised 53% of transplant patients and there was an age range of 3-17 years (mean age: 12 years). The age group that represented most of the patients (43%) was the group of adolescents 12-14 years of age (Table 1). The main causes of chronic renal failure were glomerulonephritis in 68.5% (50% chronic, 10% focal segmental, 8.5% diffuse mesangial proliferative). Moreover, hereditary causes (familial nephritis) and polycystic kidney together represented 5%. Sixty five patients (80%) received grafts from living related donors and 16 (20%) patients from cadaveric donors.
Serological Status Against CMV
The serological status of 81 recipients was available. However, only 55 complete pairs were found (donor/receptor) of IgG antibodies against CMV prior to renal transplant. Twenty pairs (25%) corresponded to the high-risk group (D+/R-); 33 pairs (41%) had a D+/R+ pattern and one pair, D-/R+ pattern (1.3%), both from the normal risk group. One pair (1.3%) had a low-risk pattern (D-/R-). Donor serology was unknown in 26 incomplete pairs; in 15 (18.5%) the recipient was positive (R+) and in 11 (13.5%) the recipient was negative (R-). The latter group was rated as high risk and was added to the D+/R- group.

Immunosuppressive Therapy
Of the patients, 97.5% (79/81) received some form of immunosuppressive therapy and two patients received no immunosuppression due to presenting hyperacute rejection after transplantation. The most commonly administered therapy was cyclosporin (CSP) (66%) combined with rapamycin, mycophenolate, and azathioprin. Other combinations included mycophenolate and rapamycin (23%). Polyclonal antibodies were administered (ATGAM) to 20/81 patients considered at high risk to develop immediate transplant rejection, of which 15/16 patients were those with cadaveric donor transplantation and 5/65 from living related donors.

Prophylactic Treatment for CMV
Of the 81 patients studied, 38 required prophylactic treatment (21 seronegative, 10 according to serology and cadaveric donor, six cadaveric donors, and one with ATGAM treatment). Two low-risk patients received prophylaxis without requirement. In 11/38 (29%) patients, prophylaxis was adequate, in five (13%) patients it was insufficient, in two (5%) incomplete, in 11 (29%) insufficient and incomplete, in 4/38 (11%) it was inferior and in five patients (13%) prophylaxis was omitted (Table 2).

When separating the indications and classifying prophylaxis according to year of transplantation, we found that the year where adequate prophylaxis was administered with greatest frequency was 2005 (25.9%), and the lowest frequency was in 2004.

Frequency of CMV Infection and Disease
In the 3 years of study, 20% (17/81) of patients analyzed were suspected to have or proven to have infection by CMV. In 11/38 (29%) patients, prophylaxis was adequate, in five (13%) patients it was insufficient, in two (5%) incomplete, in 11 (29%) insufficient and incomplete, in 4/38 (11%) it was inferior and in five patients (13%) prophylaxis was omitted (Table 2).
CMV during the first 6 months after renal transplantation. There was no gender predominance and age range was 10-16 years (mean age: 12 years). During the year 2004 we had the highest number of proven and suspected infections, together covering 70% of the cases reported during the 3 years. Of the 17 patients described, four patients (23.5%) had suspected infection, 10 (58.8%) patients had asymptomatic infection, two (12%) patients had disease, and one (6%) patient ad infection plus graft rejection (Table 3).

Table 3. Frequency of infection and disease due to CMV according to year of transplant in 17 patients

<table>
<thead>
<tr>
<th>Year of transplant</th>
<th>Suspected</th>
<th>Asymptomatic infection</th>
<th>Disease</th>
<th>Infection and rejection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>3 (17.6)</td>
<td>8 (47)</td>
<td>1 (5.9)</td>
<td>0 (0)</td>
<td>12 (70.5)</td>
</tr>
<tr>
<td>2005</td>
<td>1 (5.9)</td>
<td>2 (11.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>2006</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.9)</td>
<td>1 (5.9)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (23.5)</td>
<td>10 (58.7)</td>
<td>2 (11.8)</td>
<td>1 (5.9)</td>
<td>17 (100)</td>
</tr>
</tbody>
</table>

Clinical Description

Suspicion

Three cases with suspected CMV infection were included in the normal risk group (D+/R+) with grafts from living related donor and one patient was included in the high-risk group with cadaveric donor (D?/R-). The first three patients were found without clinical manifestations but with alterations in hematology such as lymphocytosis as well as abnormalities in liver function tests with elevated transaminases. None of the cases had confirmed CMV infection with determination of IgM or seroconversion. In these patients determination of antigenemia pp65 was not done. These events occurred from the first to the sixth month after transplantation and only one patient was treated with acyclovir. These three patients continue with their functional graft. The high-risk patient presented posttransplant general symptoms of fever, hypertension, oliguria, fatigue, weakness and decreased appetite during the first month as well as elevated creatinine, lymphopenia, thrombocytopenia and anemia. This patient received incomplete prophylaxis with i.v. gancyclovir and suffered graft loss and is undergoing hemodialysis (Table 4).

Asymptomatic Infection

There were 10 cases of asymptomatic infection, 80% in 2004 and 60% during the first 6 months after transplantation; 90% of the patients belonged to the high-risk group of which seven patients were D+/R- and two patients were D?/R-. The remaining patient was included in the normal risk group (D+/R+). Eighty percent of the grafts were living donor related and 20% were cadaveric donors. In 30% of the ten patients, prophylaxis was omitted, and 50% received inadequate prophylaxis. In two patients the diagnosis of infection was based on seroconversion, IgM+ and pp65 antigenemia positive, in one patient seroconversion and positive antigenemia were obtained, in four patients seroconversion and IgM+ and in the remaining three patients only IgM+. The majority of the patients were asymptomatic, but three patients had fever of short duration. One of these patients also presented asthenia and adynamia and another patient presented diarrhea and pain at the graft site. Two patients had lymphopenia <2000 cells/mm³. However, in most patients the percentage of lymphocytes in peripheral blood was between 50 and 60%. All patients recovered renal function after transplantation, but three patients had increased serum creatinine at the time of CMV infection. Seventy percent received appropriate treatment with i.v. gancyclovir followed by oral gancyclovir or valgancyclovir, 20% received inadequate treatment with acyclovir, and one patient was not treated. None of the patients developed disease (Table 4).

Disease

The two cases of CMV disease occurred in high-risk patients, one from a living related donor and another from a cadaveric donor, both with negative serology. Both patients received inadequate prophylaxis. The first case presented clinical data of pneumonitis manifested by productive cough, tachypnea, dyspnea and hypoxemia at 6 months posttransplant. The patient also presented hypertension with increase in creatinine to 1.9 mg/dL. There were alterations in hematology such as lymphocytosis, anemia and alteration in transaminases, confirming CMV disease based on positive IgM determination, seroconversion and positive determination of pp65 antigenemia with 54 cells positive/200,000 leukocytes. The patient was treated with i.v. gancyclovir (10 mg/kg/day) for 21 days followed by oral gancyclovir (30 mg/kg/day) for 90 days with a resulting cure.

The second case occurred in the year 2006 in a preschool 5-year-old female who received a cadaveric donor organ. Immunosuppressive therapy was administered after
Table 4. Clinical description and actual status of the 17 pediatric patients who developed suspicion, infection and disease due to CMV after renal transplant

<table>
<thead>
<tr>
<th>Year of transplant</th>
<th>Age</th>
<th>Gender</th>
<th>Donor</th>
<th>Risk</th>
<th>Transfusion</th>
<th>Prophylaxis</th>
<th>Inmunosuppres- sion</th>
<th>Diagnosis</th>
<th>Months diagnosed post transplant</th>
<th>Clinical data</th>
<th>Laborator y data</th>
<th>Treatment</th>
<th>Actual status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2004 4 F</td>
<td>Living related</td>
<td>D+/R+</td>
<td>No</td>
<td>Not required</td>
<td>CSP + rapamycin</td>
<td>Suspected</td>
<td>1</td>
<td>Asymptomatic</td>
<td>Lymphocytosis</td>
<td>Acyclovir 10 mg/kg/day x 90 days</td>
<td>Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 2004 16 M</td>
<td>Living related</td>
<td>D+/R+</td>
<td>No</td>
<td>Not required</td>
<td>CSP + myco- phenolate</td>
<td>Suspected</td>
<td>6</td>
<td>Asymptomatic</td>
<td>ALT</td>
<td>No treatment</td>
<td>Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 2004 14 M</td>
<td>Cadaveric</td>
<td>D?/R-</td>
<td>Yes</td>
<td>Incomplete</td>
<td>Mycophenolate + rapamycin + ATGAM</td>
<td>Suspected</td>
<td>1</td>
<td>Fever, SAHβ, oliguria, asthenia, lymphopenia, plateletopenia, anemia, creatinineβ</td>
<td>No treatment</td>
<td>HD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 2004 13 F</td>
<td>Living related</td>
<td>D+/R+</td>
<td>Yes</td>
<td>Not required</td>
<td>CSP + rapamycin</td>
<td>Suspected</td>
<td>5</td>
<td>Asymptomatic</td>
<td>Lymphocytosis, creatinineβ</td>
<td>No treatment</td>
<td>Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 2004 10 F</td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Insufficient</td>
<td>Mycophenolate + rapamycin</td>
<td>Infection</td>
<td>5</td>
<td>Asymptomatic</td>
<td>Gancyclovir i.v. Functional graft 10 mg/kg/day x 21 days</td>
<td>Oral gancyclovir 12 mg/kg/day x 90 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 2004 14 M</td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Omitted</td>
<td>Mycophenolate + rapamycin</td>
<td>Infection</td>
<td>11</td>
<td>Asymptomatic</td>
<td>Lymphocytosis IgM CMV, Antig pp65</td>
<td>Gancyclovir i.v. Functional graft 10 mg/kg/day x 21 days</td>
<td>Valgancyclovir 15 mg/kg/day x 90 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 2004 10 M</td>
<td>Living related</td>
<td>D+/R+</td>
<td>Yes</td>
<td>Not required</td>
<td>CSP + rapamycin</td>
<td>Infection</td>
<td>1</td>
<td>Fever, pain at graft site and diarrhea</td>
<td>Lymphocytosis, anemia creatinineβ IgM CMV</td>
<td>Gancyclovir i.v. Functional graft 5 mg/kg/day x 14 days</td>
<td>Gancyclovir i.v. 8 mg/kg/day x 90 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 2004 3 M</td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Insufficient</td>
<td>CSP + myco- phenolate</td>
<td>Infection</td>
<td>2</td>
<td>Fever</td>
<td>Lymphocytosis, anemia</td>
<td>Gancyclovir i.v. Functional graft 12 mg/kg/day x 21 days</td>
<td>Oral gancyclovir 60 mg/kg/day x 70 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Clinical description and actual status of the 17 pediatric patients who developed suspicion, infection and disease due to CMV after renal transplant.

<table>
<thead>
<tr>
<th>Year of transplant</th>
<th>Age</th>
<th>Gender</th>
<th>Donor</th>
<th>Risk</th>
<th>Transfusion</th>
<th>Prophylaxis</th>
<th>Immunosuppression</th>
<th>Diagnosis Months diagnosed posttransplant</th>
<th>Clinical data</th>
<th>Laboratory data</th>
<th>Treatment</th>
<th>Actual status</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 2004 12 M</td>
<td></td>
<td></td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Insufficient</td>
<td>CSP + mycophenolate</td>
<td>Infection 8</td>
<td>Asymptomatic Lymphocytosis IgM CMV</td>
<td>Oral valganclovir 7 mg/kg/day x 120 days</td>
<td>Functional graft</td>
<td></td>
</tr>
<tr>
<td>10 2004 13 F</td>
<td></td>
<td></td>
<td>Cadaveric</td>
<td>D?/R-</td>
<td>Yes</td>
<td>Insufficient</td>
<td>CSP + rapamycin + ATGAM</td>
<td>Infection 10</td>
<td>Asymptomatic Lymphocytosis IgM CMV</td>
<td>Valganclovir Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 2004 10 F</td>
<td></td>
<td></td>
<td>Cadaveric</td>
<td>D+/R-</td>
<td>No</td>
<td>Omitted</td>
<td>Mycophenolate + rapamycin</td>
<td>Infection 2</td>
<td>Asymptomatic Lymphocytosis Anemia IgM CMV</td>
<td>Oral acyclovir Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 2004 5 M</td>
<td></td>
<td></td>
<td>Living related</td>
<td>D?/R-</td>
<td>No</td>
<td>Omitted</td>
<td>CSP + mycophenolate</td>
<td>Infection 2</td>
<td>Asymptomatic Lymphocytosis Anemia IgM CMV</td>
<td>Gancyclovir i.v. Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 2005 13 M</td>
<td></td>
<td></td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Inferior</td>
<td>CPM + azathioprin</td>
<td>Infección 5</td>
<td>Fever, asthenia, adenemia Creatinine Beta, Anemia, IgM+ CMV</td>
<td>Gancyclovir i.v. Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 2005 13 M</td>
<td></td>
<td></td>
<td>Living related</td>
<td>D+/R-</td>
<td>No</td>
<td>Adequate</td>
<td>CSP + azathioprin</td>
<td>Infection 12</td>
<td>Asymptomatic Lymphocytosis IgM CMV</td>
<td>No treatment Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 2004 15 F</td>
<td></td>
<td></td>
<td>Living related</td>
<td>D+/R-</td>
<td>Yes</td>
<td>Incomplete</td>
<td>Mycophenolate + rapamycin</td>
<td>Disease, pneumonitis x CMV 6</td>
<td>SAH, dyspnea, hypoxemia, cough, pneumonitis due to CMV</td>
<td>Gancyclovir i.v. Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 2006 5 F</td>
<td></td>
<td></td>
<td>Cadaveric</td>
<td>D?/R-</td>
<td>No</td>
<td>Insufficient &amp; incomplete</td>
<td>CSP + mycophenolate + ATGAM</td>
<td>Disease viral symptoms 6</td>
<td>Fever, oliguria, pain, asthenia, adynamia, hypoxemia, myalgias</td>
<td>Gancyclovir i.v. Functional graft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 2005 16 F</td>
<td></td>
<td></td>
<td>Cadaveric</td>
<td>D?/R-</td>
<td>No</td>
<td>Insufficient &amp; incomplete</td>
<td>CSP + rapamycin + ATGAM</td>
<td>Infection + rejection 7</td>
<td>Asthenia, adynamia, hypoxemia, oliguria, vomiting</td>
<td>Gancyclovir 3 mg/kg/day x 16 days</td>
<td>Graft loss HD</td>
<td></td>
</tr>
</tbody>
</table>

CMV, cyclosporin; CPM, cyclophosphamide; HD, hemodialysis; SAH, systemic arterial hypertension; ALT, alanine aminotransferase, AST, aspartate transaminase.
transplantation with ATGAM. Prophylaxis was insufficient and incomplete with i.v. gancyclovir (5 mg/kg/day) for 21 days followed by oral acyclovir. In the sixth month after transplantation the patient presented nonspecific clinical symptoms corresponding to CMV viral syndrome with presence of malaise, fever 39°C, fatigue, weakness, and muscle and abdominal pain. Oliguria was present with elevated creatinine (1.3 mg/dL) and alterations in blood count with the presence of lymphocytosis, neutropenia and anemia. Diagnosis was made based on seroconversion and IgM+ determination. The patient was treated with i.v. gancyclovir (10 mg/kg/day for 21 days) followed by oral valgancyclovir (30 mg/kg/day) with the time of administration unknown (Table 4).

CMV Infection and Graft Rejection
A high-risk (D/R-) adolescent female (16 years of age) who received a cadaveric donor organ presented in the year 2006. Immunosuppressive therapy was administered with ATGAM after transplantation. CMV prophylaxis was incomplete, initially with i.v. gancyclovir followed by oral valgancyclovir. During the seventh month after transplantation, the patient demonstrated oliguria, fatigue, weakness, vomiting and decreased appetite, nitrogen elevation and mild anemia, attributing these symptoms to a CMV infection with positive IgM and seroconversion. She was treated with gancyclovir (3 mg/kg/dose) for 16 days; however, there was graft loss. The renal biopsy was reported as chronic graft rejection (Table 4).

Risk Factors Associated with the Development of CMV Infection or Disease
Cases reported were those of the 13 patients who had as the final diagnosis that of infection or disease plus rejection due to CMV infection. Controls included the 53 patients who did not develop infection or disease during the follow-up period. Not included in the analysis were four patients with suspected diagnosis of CMV infection and 11 patients who experienced rejection during the first month posttransplantation due to the fact that the time for developing infection by CMV was very short. Based on the lesser probability of having had a prior CMV infection, it was decided to take as a risk factor those patients younger than the mean age. Due to the greater frequency of inadequate prophylaxis (44%) during the year 2004, having been transplanted in the specified year was used as a risk factor. In most cadaveric donors the serological status for CMV is unknown and the recipients receive additive immunosuppressive therapy with ATGAM, so this was taken as a risk factor. The possibility of CMV transmission through transfused blood products has been described and for this reason blood transfusion was noted as a risk factor. Table 5 shows the results obtained in the bivariate analysis: the seronegative status for CMV of the recipient, being transplanted in the year 2004 and inadequate prophylaxis for CMV were the only risk factors in which statistical significance was obtained.

Multivariate analysis was performed using logistic regression analysis in a saturated model, including all independent variables described in the bivariate analysis. The only variables found to be independently associated with the development of infection were seronegative status for CMV of the recipient before transplantation, as well as performing the transplant in 2004. However, the latter factor is only in regard to the local environment due to the characteristics of our hospital. Therefore, in a second multivariate analysis, excluding this last variable (year of transplantation), only the serological status was obtained as an independent risk factor associated with the development of infection or disease as significant with an OR of 41.0 (95% CI 4.82-348 135, p = 0.001) (Table 6).

DISCUSSION
CMV infection represents one of the most important causes of infection or disease between the first and sixth month post-renal transplantation, with an estimated incidence of 30-78%. This infection rate varies according to the seroprevalence for CMV in different countries, reported to be from 40-80% of seropositivity in the general population.9 This seroprevalence has a close relationship with the serological risk prior to renal transplant in a group of patients. In adult subjects, in our environment a seroprevalence of 90% has been reported.19,20 However, in our study it was found that only 60% of the recipients had been in contact with CMV. It is probable that this prior immunity offered protection to some of the study subjects, thereby resulting in a frequency of infection or disease of 20% and of only 2.5% of disease, which is less than reported in prior studies.4,17 Also, although in seronegative children this frequency of infection was doubled (40%), it continues to be lower to what is reported worldwide for individuals with
### Table 5. Risk factors for developing infection or disease in 13 cases and 53 controls

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cases (n = 13)</th>
<th>Controls (n = 53)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV receptor negative</td>
<td>12 (92.3%)</td>
<td>12 (22.6%)</td>
<td>41</td>
<td>4.82-348.13</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>Inadequate prophylaxis</td>
<td>11 (84.6%)</td>
<td>9 (16.98%)</td>
<td>26.88</td>
<td>5.06-142</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>Year 2004</td>
<td>9 (69.2%)</td>
<td>12 (22.6%)</td>
<td>7.68</td>
<td>2.00-29.4</td>
<td>0.004*</td>
</tr>
<tr>
<td>Cadaveric donor</td>
<td>4 (30.8%)</td>
<td>5 (9.4%)</td>
<td>4.26</td>
<td>0.95-19.03</td>
<td>0.122*</td>
</tr>
<tr>
<td>Age &lt;11 years</td>
<td>6 (46.15%)</td>
<td>17 (32%)</td>
<td>0.55</td>
<td>0.16-1.89</td>
<td>0.39*</td>
</tr>
<tr>
<td>Male</td>
<td>7 (53.8%)</td>
<td>26 (49%)</td>
<td>1.21</td>
<td>0.35-4.08</td>
<td>1*</td>
</tr>
<tr>
<td>Packed red blood cell transfusion</td>
<td>3 (23%)</td>
<td>19 (35.8%)</td>
<td>0.54</td>
<td>0.13-2.19</td>
<td>0.58*</td>
</tr>
<tr>
<td>Administration of ATGAM</td>
<td>3 (23%)</td>
<td>7 (13.2%)</td>
<td>1.97</td>
<td>0.43-8.97</td>
<td>0.65*</td>
</tr>
</tbody>
</table>

*Mantel-Haenszel $\chi^2$.

### Table 6. Statistically significant risk factors for developing infection or disease due to CMV in transplanted patients

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV receptor negative</td>
<td>109.86</td>
<td>6.96-1733.89</td>
<td>0.001*</td>
</tr>
<tr>
<td>Year 2004</td>
<td>25.44</td>
<td>2.52-256.17</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*Mantel-Haenszel $\chi^2$.

CMV, cytomegalovirus; OR, odds ratio; CI, confidence interval.

It is known that adding polyclonal antibodies to immunosuppressive therapy causes a prolonged lymphopenia and suppression of CD4 for several months to years due to cytotoxic antibodies directed against a variety of T-cell markers. In addition to that, the same posttransplant inflammatory response leads to a release of proinflammatory cytokines that can activate latent CMV infection of the transplanted organ and increase the frequencies of CMV infection.\(^{21}\) However, in our study, only 20% of patients who received ATGAM developed an infection, perhaps because 60% of these patients had protective antibodies against CMV prior to transplant which, in some way, protected the patients receiving this biological compound.

It still remains to be demonstrated in the literature whether the mere fact of receiving cadaveric donor graft may increase the risk of CMV infection or disease. It has only been demonstrated that these patients require a higher degree of immunosuppressive therapy, increasing the risk of developing CMV infection or disease because of the higher state of immunosuppression to which the patient is subjected.\(^{24}\) In a study of 72 pediatric renal transplant patients, a relationship between CMV infection and receiving cadaveric graft was not found. In our study, this feature had a marginal value for the development of CMV infection (0.96–16). However, it was not significant ($p = 0.169$), partly due to the proper administration of prophylaxis or to an early graft failure from causes other than CMV infection.

It is possible that the sum of the various risk factors found in some patients increases the risk for developing CMV infection or disease. In our study, 5/17 infected patients (30%) had three or more risk factors: they were

...
seronegative for CMV and had cadaveric graft. Four of these patients received ATGAM. In all of these patients, inadequate drug prophylaxis administration was documented.

Moreover, it has been shown that immunological renal transplant rejections are more frequent in the male population (only according to frequency). However, in our study there was no statistically significant difference between genders or according to age or transfusions administered.

The clinical condition resulting in CMV infection in the group of patients studied is variable. Asymptomatic infection occurred in the majority of patients and only 10% of those patients infected had clinical data with disease. This is consistent with previous reports by Smith et al. who mentioned that the clinical course of a primary infection does not differ from the reactivation or superinfection, with the majority of cases having an asymptomatic clinical presentation, coinciding with the results of our study.

Disease due to CMV refers to the acute symptomatic infection or tissue invasion by CMV, initially being an asymptomatic infection that evolves to CMV syndrome (leukopenic fever and increase in CMV antigen titers) and invasive CMV disease (pneumonitis, hepatitis and gastrointestinal disturbances). Some histopathological studies show that the disease associated with CMV is primarily located in the transplanted organ and later disseminates systemically, causing pneumonitis, enteritis and hepatitis. However, the literature mentions a reduction of 0-5% disseminated CMV disease on administration of prophylactic therapy after renal transplant. This reduction, however, was not able to be established in our study, initially due to the study design and because the recommendation for prophylaxis administration in high-risk patients was, for the most part, carried out in inadequately in 27/38 (71%) patients. For this reason we were unable to clearly establish this protective factor.

Currently, diagnosis of CMV infection is based on the determination of viral genetic material or on the identification of viral antigens on the early production of leukocytes during the infection.

This study has some limitations. In addition to its retrospective nature, diagnosis was based mainly on serological determinations of the immune response (76.4%), and in a few cases was the identification of viral antigens mentioned (17.6%).

Prior to 2006, in our hospital, there was no systematized follow-up of CMV infection. Also, there was no drug available with an adequate activity and bioavailability against CMV that would allow outpatient follow-up of transplanted patients. In this study, most of the infections were presented during 2004. It was noted that the frequency of adequate prophylactic administration was very low and, although it improved during subsequent years when an analysis was performed to determine if there was any difference, it was not statistically significant. Although all patients who were diagnosed with acute infection were high risk, there is no explanation as to why 15/25 remaining patients in this group who did not receive adequate prophylaxis were not infected. The main limitation of the study is its retrospective nature. Detailed follow-up was not documented in the medical records. There was no basis to rule out infection in patients who were suspect, confirmatory test in patients with positive serology or total dose of antiviral administered (in some infections). It is possible that the suppressive immunotherapy administered in 2004 caused greater immunosuppression in patients or that the true frequency of infection was overestimated. From 2005, the number of patients with infection apparently decreased, but it is also possible that the diagnosis improved and confirmatory tests were requested prior to treatment initiation on asymptomatic patients.

There is an ongoing debate about the optimal management of a patient with renal transplantation. Most publications seem to favor the administration of prophylaxis in high-risk patients and early therapy in those at low risk. Data analyzed here show that omission of prophylaxis, administration of inappropriate drugs or insufficient time or dose increases the risk of infection, especially in high-risk subjects.

Finally, standardized follow-up for all patients with sensitive and specific virological tests at determined intervals and initiation of antiviral treatment in a timely manner will allow for reduction of the frequency of CMV disease in these types of patients. Only a prospective study will answer these questions and establish the actual frequency of CMV infection in renal transplant patients.

REFERENCES


Suckling behavior at 48 hours of life in low and normal birth weight infants and relationship with growth at 28 days of life

Mario Enrique Rendón-Macías, Héctor Domínguez-Jiménez, Yolanda Aguilar-Álvarez

ABSTRACT

Background. Inadequate suction may affect newborn (NB) growth, especially in low birth weight (LBW) infants. The aim of this study was to determine the relationship between suckling efficacy at 48 h of life with neonatal weight gain (WG) in infants exclusively breastfed with normal birth weight (NBW >2.5 kg) and LBW (≤2.5 kg).

Methods. We carried out a cohort study in healthy NB rooming with their mothers in hospital. At 45-48 h after birth, prior to discharge and after being weighed, suckling efficacy was assessed using the ECLES scale by a previously trained physician. At 28 days of life, WG was assessed. We included only those infants who continued to be exclusively breastfed.

Results. There were 80 NB, 51 (63.7%) with NBW and 29 (36.3%) with LBW; 47 (58.7%) NB with normal suckling (ECLES 39-40), 24 (30%) with mild suckling impairment (ECLES 37-38), and nine NB (11%) with moderate suckling impairment (ECLES 32-36). There was a relationship between WG and suckling efficacy, most noticeable in LBW infants [WG for newborns with NBW and normal suckling = 1169 ± 222 g, mild impairment of suckling = 995 ± 257, and moderate impairment of suckling = 1073 ± 245; for LBW infants and normal suckling = 911 ± 229, mild impairment of suckling = 1010 ± 299, and moderate impairment of suckling = 460 ± 115. ANOVA ECLES* weight F = 3.8, p = 0.04, FECLES = 1.5, p = 0.39 and Fweight = 4.5, p = 0.12]. There were no differences in parity, gestational age, gender, and nipple condition of the mothers among infants, with or without normal sucking.

Conclusions. Mild or moderate sucking impairment after 48 h of life was associated with lower weight gain during the neonatal period, especially in LBW infants. Oral stimulation therapy is suggested in these children before discharge and with close follow-up.

Key words: suckling assessment, weight growth, infants, low birth weight.

INTRODUCTION

Breast milk is the optimal feeding method for newborns (NB) and infants because of its bioactive properties that facilitate the transition between intra- and extrauterine life. The World Health Organization (WHO) recommends that infants be fed exclusively on breast milk from birth to 4–6 months or until 1 year of age.1

The neonate should initiate breastfeeding within 1 h of life. In order to accomplish this there should be anatomic integrity of the oral cavity and proper oral motor development. Both are acquired from 34 weeks of gestational age and are characterized by rhythmic and coordinated movements.2 However, there are factors that interfere with early onset of lactation. These include cesarean birth, maternal history of epidural block, severe diseases of the newborn, low birth weight (LBW) and prematurity. According to Nakao and Moji, in Japan, the elapsed time from birth to the onset of lactation may influence early abandonment of breastfeeding, i.e., those who began breastfeeding between 30 min and 2 h of life were breastfed less time than those who started in the first 30 min.3 In another study by Giannakou et al. in a hospital in Athens, Greece with 312 children, it was found that the initiation of postpartum breastfeeding favored breastfeeding in 85%; however, at 40 days of age, the prevalence decreased to 35%. Among the causes of abandonment, the most frequent was associated with the perception of having insufficient milk in 36% of the mothers. In addition, 2% of mothers said they observed frequent choking of their child.4
There are international guidelines to ensure the success of breastfeeding. The main guideline is the promotion during the prenatal stages by healthcare personnel, which includes responding to questions the mother may have and educating her on the nutritional value of breast milk. In the second trimester an evaluation should be made of the characteristics of the nipples for detecting any abnormalities (flat or inverted nipples), which could reduce the efficiency of a suckling infant. During the postpartum period, breastfeeding should begin as soon as possible and should be on demand, offering the breast 8-12 times a day, without night time suspension as well as recommending to the mother to alternate breasts when feeding so as to improve complete emptying of both breasts.\(^5\)

Curran and Barness, referring to an adequate supply of breast milk, stated that when satisfied after each feeding, the infant sleeps 2-4 h between feedings and gains weight adequately. Furthermore, these authors add that breastfeeding can be considered to progress satisfactorily if the NB does not lose any weight beyond the fifth day of life and continues gaining weight between days 12 and 14.\(^6\)

In the postpartum stage, NB suckling on the areola is the most important stimulus for milk production. This promotes the secretion of prolactin, responsible for the production of milk and is regulated by the central nervous system. Initially, milk production in the immediate postpartum period is up to 150 mL but is significantly modified by local factors that are dependent on emptying of the breasts.\(^7\) Meanwhile, oxytocin released from the posterior pituitary gland through a neuroendocrine reflex caused by stimulation of sensory nerve endings in the areola during suckling stimulates myoepithelial cells of the alveoli of the breast. During correct lactation the infant introduces the nipple into the mouth and, in large part, the areola such that a long nipple is formed that reaches almost to the child’s soft palate. Milk is then extracted, not by the strength of the suction, but by the milking movement of the tongue against the hard palate.\(^8-10\)

Maternal factors related to early cessation of exclusive breastfeeding have been studied, such as cultural, economic, occupational, maternal health, inadequate prenatal promotion of breastfeeding and low milk production.\(^10\) Among the factors related with the NB, consideration has been given to LBW, little or no weight gain in the first 5 days of life, existence of a serious illness and poor suction.\(^11\) Of these, low weight gain has been mainly associated with early abandonment of breastfeeding because low growth usually leads the mother to consider a dietary supplement with infant formula or even total replacement.

It has been accepted that a healthy NB develops effective suction in order to grow and develop. In contrast to this, the study by Mizuno and Ueda proposed that children with altered suction during their first months of life confirmed an abnormal neurodevelopment in later months.\(^12\) These authors concluded that suction status during the neonatal period may be an early indicator of neurological damage.

In various studies, in term as well as premature NBs, oral stimulation for optimal suction has shown an improvement in the clinical conditions of growth of the NB and infants. Therefore, it is believed that the evaluation of suction is of vital importance as it allows for reinforcement for the mother on the technique of suction and, if necessary, to initiate a timely intervention directed to improve better suction by the NB.\(^13,14\) To this end, clinical evaluations have been developed that objectively allow evaluation of adequate suction. One of these evaluations was proposed by Jensen et al. and evaluates five elements: attachment of the baby to the breast, audible swallowing, shape of nipple, type of breast and support of the baby by the mother (by themselves or if they required assistance). This scale is graded on points. The highest score is 9-10 and indicates a successful breastfeeding with minimal assistance. A score of 4-5 (low) indicates the need for assistance and possible feeding difficulty.\(^15\) In Mexico, the Clinical Scale for Evaluation of Suction (ECLES) is used,\(^16,17\) which was designed with the goal of being used as a clinical tool for daily evaluation of the conditions of suction of a NB. This scale explores the three areas of suction: expression/suction, swallowing and respiration and integrates the clinical manifestations of each of these. It has been previously validated and shown a high internal and interobserver consistency with simple training, in addition to not requiring special equipment for its application. It also takes into consideration the most important signs to be aware of during the process of suction and allows for evaluation of the phases of coordination that translate the effectiveness of the suction to ensure a satisfactory volume and without uncoordinated data.\(^16\) The scale is scored from 4 to 40. In term healthy children, it has been determined that >95% have scores between 39 and 40, whereas children with serious suction-swallowing-breathing problems...
have scores <32. Scores of 32-38 were found in NBs with mild to moderate alterations. Currently, suction evaluation is not systematized at the time of birth nor has it been determined if its alterations could have an influence on the efficacy of suction as well as on growth during the first month of life. The aim of this study was to determine whether there is a correlation between the effectiveness of suction at 48 h of life and weight growth per month of life in infants with adequate weight (AW) and low weight (LW) at birth (<2500 g) with predominant breastfeeding.

Subjects and Methods
A longitudinal study of two cohorts was carried out. One was comprised of NBs with AW (>2.5 kg) according to WHO criteria and the other cohort was comprised of NBs with LW (≤2.5 kg) at birth. All NBs were born in the Rural Hospital Oportunidades N° 80, de Mapastepec, Chiapas and in the Rural Hospital Oportunidades N° 43, Huautla de Jiménez, Oaxaca, IMSS. Selected for the study were NB of >37 weeks of gestation determined by date of last maternal menstruation and confirmed by Capurro evaluation, born vaginally or by cesarean section, without data of neonatal asphyxia (Apgar 8-10 at 5 min), and without cardiac, respiratory, neurological or GI diseases. All infants were rooming with the mother and all had >45 h since birth, as well as having initiated breastfeeding. To participate, the mother had to declare that she wished to or intended to breastfeed the child for at least 6 months. Only those NBs whose breastfeeding was confirmed were included. To achieve the study objectives, it was established as criteria analysis that only neonates who were exclusively breastfed up to 28 days of life (second evaluation) be included as well as evidence of being disease free. Prior to study initiation, the protocol was approved by the Local Committee of Health Research, HP, CMNS XXI, IMSS. In all cases, written informed consent was requested and anonymity and confidentiality of the data were maintained. Observation of suction during feeding of the NB was performed individually, protecting the privacy and dignity of the mother.

Procedures
After written acceptance of the parents or guardians and, at least <2 h of fasting, we performed comprehensive physical assessment of the infants. The following information was recorded: gestational age, gender, anthropometry at birth, Apgar score and type of delivery. Information was obtained from the mothers in regard to history of infections and exposure to medications during gestation, smoking and alcohol consumption. Maternal age was also determined and data in regard to their nipples (formed, flat or inverted.)

All infants selected were weighed without clothing using a NB electric digital precision scale (SECA 5345, reading scale: 10 g) to determine the weight in grams before initiation of feeding. Height was determined with an infantometer. Cardiac and respiratory stability were confirmed as well as confirming a normal neurological examination.

Evaluation of suction effectiveness was performed by personnel previously trained with support from the ECLES scale. This scale was previously validated and consists of ten items that assess the suck-swallow-breathe coordination with the use of clinical data. Each item is spelled out in four options. The minimum score is 10 and the maximum is 40. Intraobserver concordance index is kappa = 0.95 (95% CI = 0.9-1.0). The evaluation was conducted by direct observation of the breast suction by the evaluator during a 15-min period. For each infant, two feedings were evaluated with an interval of at least 2 h and the average of the evaluations was analyzed. According to the validation of the scale, effective suction was considered to be one with a score of 39 and 40, mild impairment with 37-38 points, mildly abnormal with 36-20 points and severe with <20 points.

Patients who completed the first phase then were given an appointment between days 28 and 35 of life for a well-baby visit. For this second phase, the mothers were required to bring the NB fasting for <2 h. Evaluation of the effectiveness of the suction and weight was carried out under the same conditions as discussed above. Also, in this second review, the mother was questioned about her infant’s behavior during feedings, especially in regard to the presence of regurgitation, vomiting, choking or gagging, and the existence of illness, hospitalizations and medications.

Statistical Analysis
Data collection was performed on previously designed formats and stored in an electronic database. Simple frequencies and percentages were obtained for nominal and quantitative variables summarized as averages and maximum and minimum values. Mean and SD were obtained.
for weight, with a normal distribution. For comparisons of proportions between groups as classified by ECLES in regard to the suction efficiency, Pearson $\chi^2$ test was used and for quantitative measures nonparametric Kruskal-Wallis test was used.

To analyze differences in weight gain according to groups of suction condition, and in order to observe the possibility of interaction with birth weight, analysis of variance (ANOVA) adjusted for gender, WG and characteristics of mother’s nipples was performed. When there were significant differences, groups were compared according to their birth weight and condition of the suction efficiency through post-hoc Bonferroni test. For all statistical tests, $p < 0.05$ was considered statistically significant. Analyses were performed using the statistical package SPSS v.18.

RESULTS

From a total of 122 NBs, 80 met the selection criteria, 51 had AW at birth and 29 LW. Average gestational age for the entire group was 38 weeks and there was a predominance of males (46 compared with 34 females, ratio 1.91:1). There were no statistically significant differences between genders and their weights according to the groups of analysis (AW and LW at birth).

At the time of assessment of the effectiveness of the suction, 47 infants showed normal suction (58.7%), 38 showed mild alterations (47.5%), and nine (11.3%) showed moderate alterations (Table 1). No neonate was rated as having abnormal suction so severe that it required a change in type of feeding. Although most NB were born by vaginal delivery, the highest frequency of infants with low scores on the ECLES scale was observed in those born by cesarean delivery.

Evaluation at 48 h of Life

At 48 h of life, 80 NB remained with exclusive breastfeeding in shared accommodations. None had any data of additional disease. Exploration of the condition of the mother’s nipples showed a slightly higher proportion of changes in the group of infants with low ECLES scores, without a statistically significant difference. It is notable that the median weight at 48 h was lower for the ECLES group with a score of 39-40 points (Table 1).

Evaluation at 30 Days of Life

At this time of follow-up, all infants maintained exclusive breastfeeding. Table 2 shows the growth characteristics of the weight of the NB according to their birth weight and ECLES score. A lower weight gain (g) was observed at 1 month of life in patients with low ECLES scores and average daily gain. This was more affected in patients with LBW who had an average increase of 460 g. In general, infants with LBW showed less weight gain than those born with AW (Figure 1). This difference remained between infants with normal AW and LW suction ($p < 0.005$), but not for infants with mild impairment. In cases of moderate impairment the different was significant in weight gain ($p < 0.001$). The same phenomenon was found when considering the average daily weight gain. When performing the ANOVA, we found that there was interaction between the greater impairment of suction and history of LW at birth, that is, when both conditions were present, the average weight gain did not reach the minimum of 25 g/day. According to information obtained from the mothers on the feeding behavior of their children, only four infants (5%) had an occasional regurgitation. It should be noted that two of them were from LW group and had moderate suction impairment.

DISCUSSION

To the best of our knowledge, this is the first study to report on the possible impact of the suction condition of a healthy newborn in regard to early weight gain. Those infants with adequate suction (assessed by the ECLES scale) according to the cohort of adequate weight as well as in low weight infants, showed an average daily weight gain higher than those with mild to moderate impairment of suction.

Breastfeeding of a NB is established during the first days of life. At birth, a term neonate has integrated the sucking and swallowing reflex that allows the child to begin oral feeding. However, the success of prolonged lactation depends on the bonding of the child with the mother. Under favorable conditions, the mother stimulates the infant to initiate breastfeeding and the infant responds to her expectations when latching on to her breast and obtaining sufficient milk for nutrition and satisfaction. Previous studies have extensively explored the maternal factor in the initiation and continuation of breastfeeding, but the NB factor has been scarcely evaluated.
Within the first 3 days of life, the infant improves suction during breastfeeding. This is one of the advantages of rooming-in, which encourage close contact between mother and child and helps to improve the flow of milk as well as bonding with the infant.

It is anticipated that at 72 h of life the infant has experienced sufficient encounters with the mother to have an effective suction for feeding. As noted in this study, even healthy NB with adequate weight can experience ineffective suction at 48 h of life, which may be associated with growth, although with adequate weight this can be lower than that found in children with strong suction.

Different authors have shown a decrease in weight in the first 2 to 5 days and in some NBs up to 1 week after birth. However, all authors agree on a weight gain from the 8th day, with recovery of the birthweight in ~90% of NBs during the second week of life. Less than 5% remain below their weight at birth or lose >10% of their weight during this decrease and are considered to be at high risk for malnutrition. Lack of weight gain tends to be attributed to ineffective breastfeeding, oftentimes explained by “an insufficient milk production.” In general, in these NBs the pattern of suction is not evaluated and therefore stimulation therapies are not done, which many have proven to be useful. In this group of NBs it was interesting to demonstrate a relationship between growth and suction efficiency.

The effect of a moderately impaired suction and growth less than that recommended of 25 g/day or more was more notable. Despite these findings, it is important to consider that the number of NBs identified to have problems with suction was limited in order to determine with certainty if inadequate suction during the first days of life could result in lower weight gain during the first month of life.

The main strength of this study is its design because it was a prospective study without any losses to follow-up. Also, the quality was assured when only infants who were exclusively breastfed were included and who were

Table 1. Characteristics of NB in accordance with the ECLES scale at birth and at 4 h of life

<table>
<thead>
<tr>
<th>Characteristics of the neonates</th>
<th>Points of ECLES scale</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39-40</td>
<td>37-38</td>
</tr>
<tr>
<td></td>
<td>n = 47</td>
<td>n = 24</td>
</tr>
<tr>
<td>Weeks of gestational age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>medin (min-max)</td>
<td>38 (37-41)</td>
<td>38 (37-41)</td>
</tr>
<tr>
<td>Birthweight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AW n (%)</td>
<td>25 (49)</td>
<td>19 (37.2)</td>
</tr>
<tr>
<td>LW n (%)</td>
<td>22 (75)</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>Weight at 48 h (g) median</td>
<td>2480 (1950-3760)</td>
<td>2690 (2000-3300)</td>
</tr>
<tr>
<td>Length at 48 h (cm) median</td>
<td>48 (41-53)</td>
<td>48 (41-52)</td>
</tr>
<tr>
<td>Males feasibility (%)</td>
<td>28 (59.6%)</td>
<td>11 (45.8%)</td>
</tr>
<tr>
<td>Vaginal delivery frequency (%)</td>
<td>39 (83%)</td>
<td>17 (70.8%)</td>
</tr>
<tr>
<td>Maternal data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 (13-39)</td>
<td>23 (17-32)</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>2 (1-7)</td>
<td>2 (1-6)</td>
</tr>
<tr>
<td>Nipple condition n (%)</td>
<td>36 (76.6)</td>
<td>19 (79.2)</td>
</tr>
<tr>
<td>Both normal</td>
<td>5 (10.6)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>One with alterations</td>
<td>6 (12.8)</td>
<td>4 (16.7)</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test.
**Pearson $\chi^2$ test.

ECLES, Clinical Scale for Evaluation of Suction; min, minimum; max, maximum.
evaluated by two physicians previously trained in suction measurement and weight of NBs. The follow-up period was short, eliminating the possibilities of acute diseases, introduction of complementary foods or other supplements. Another notable point is that as a criterion for inclusion, only NBs with sufficiently acceptable suction were accepted so as to avoid early complications, which may explain the low population of children with failure to thrive.

The study was carried out under highly conducive environmental conditions for predominantly or exclusively breastfeeding due to the close coexistence between mother and NB. Also, care was taken to include only children with initiation and continuation of maternal breastfeeding during hospitalization in order to avoid patients with diseases during lactation. This has occurred in other studies where problems of dehydration associated with breastfeeding are present during the first 24-72 h of life.

Although the number of patients was sufficient to determine statistically significant differences between extreme groups, differences found were between groups with mild or moderate weight impairment. This study evaluated suction at discharge or 48 h after birth. This period was selected to evaluate the condition closest to actual after the initiation of breastfeeding. It was considered a reasonable

### Table 2. Growth of the NB according to points of ECLES scale and weight condition at birth

<table>
<thead>
<tr>
<th>Weight gained at 28 days of life (g)</th>
<th>ECLES points</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39-40 (n = 47)</td>
<td>37-38 (n = 24)</td>
</tr>
<tr>
<td>NB with AW at birth average (SD)</td>
<td>1169 (222)</td>
<td>995 (257)</td>
</tr>
<tr>
<td>NB with LW at birth average (SD)</td>
<td>911 (229)</td>
<td>1010 (299)</td>
</tr>
<tr>
<td>All NB average (SD)</td>
<td>1032 (254)</td>
<td>998 (259)</td>
</tr>
</tbody>
</table>

Average daily weight gain during the first 28 days (g/day)

| NB with AW at birth Average (1 SD) | 38.9 (7.4)    | 33.1 (8.5)    | 32.8 (6.6)    | 35.7 (8.2)  |
| NB with LW at birth average (1 SD) | 30.4 (7.3)    | 33.6 (9.9)    | 15.3 (5.2)    | 29.9 (8.4)  |
| All NB average (1 SD)              | 34.4 (8.5)    | 33.3 (8.6)    | 28.9 (9.7)    | 33.5 (8.7)  |

NB, newborn; AW, adequate weight; LW, low weight.

ANOVA for both categories.

Weight* ECLES F =3.28 (2 gl) p = 0.04; weight F = 4.5 (1 gl), p = 0.12, ECLES F=1.51, p = 0.39.

Adjusted for gender, weeks of gestational age, and nipple characteristics of the mother.

**Figure 1.** Average daily growth according to birthweight and suction evaluation with ECLES. ANOVA, F = 5.18, gl 74-5, p <0.001; post-hoc Bonferroni. ECLES group (39-40) and ≥2500 g vs. ECLES group (39-40) and <2500 g, p = 0.005 vs. ECLES group (≤36) and <2500 g, p = 0.001. ECLES group (≤36) and <2500 g vs. ECLES group (39-40) and ≥2500 g, p = 0.005 vs. ECLES group (37-38) and ≥2500 g, p = 0.04 vs. ECLES group (≤36) and ≥2500 g, p = 0.09 vs. ECLES (39-40) <2500 g, p = 0.15 vs. ECLES group (37-38) and <2500 g, p = 0.09.
time to more precisely determine whether the infant may have early problems attaining successful breastfeeding. However, current behaviors in many hospitals are directed to early discharge, i.e., prior to 24 h. It is possible that during this period more infants may have a mildly or moderately impaired suction which, if not detected, may result in no actions taken to improve the condition.

With the evidence so far found, the need to continue suction evaluation of all NB is considered to be a necessity. This action, although part of routine hospital care, is not so common that it is routinely performed in a standardized manner. Evaluation remains at the discretion of the evaluator who oftentimes may be a maternal-child care nurse. Although the evaluation is not an absolute indicator of adequate weight gain, it may be a warning sign for following more closely the NB or infant. In particular, children at high risk for failure to thrive, such as NBs with LW or those NB with other congenital diseases (cleft palate, congenital heart disease, etc.) could manifest data of inadequate suction. In these NBs discharge may be delayed, after performing a thorough evaluation of suction and successful maternal bonding.

Discontinuation of nursing due to a "sense of insufficient milk" should not be surprising. Milk production is related to extraction. Infants with inadequate suction usually reject the mother’s breast, cry during feeding, sleep or vomit, among other signs. These mothers, according to their own choice or to indications of healthcare personnel, tend to initiate infant formula even when breastfeeding could be improved with sensory, oral, or motor therapy or support for the child.

In conclusion, we may state that there is a correlation between suction efficiency and weight gain of breastfed term NBs. Assessment of suction characteristics for all infants should be carried out within the first 48 h after birth. When cases with impaired suction are detected, early measures for stimulation should be provided.

Acknowledgments
The authors appreciate the help of the medical personnel and the participating mothers of the clinics of the Hospital Rural Oportunidades N° 80 de Mapastepec, Chiapas, and Hospital Rural Oportunidades N° 43, Huautla de Jiménez, Oaxaca, Instituto Mexicano del Seguro Social.

Funding for the study was provided by Consejo Nacional de Ciencia y Tecnología (CONACYT) (Apoyo Complementario a Investigadores en Proceso de Consolidación) with registration #0090246.

REFERENCES


Usefulness of C-reactive protein in the diagnosis of bacterial infection in the pediatric patient with cancer, fever and neutropenia

Martín Penagos-Paniagua,1 Miguel Ángel Villasis-Keever,2 María Guadalupe Miranda-Novales,1 Andrea Tapia-Marcial,3 Hugo Rivera-Márquez,4 Roberto Bernaldez-Ríos,5 Enrique Lopez Aguilar,4 Fortino Solórzano-Santos1

ABSTRACT

Background. Diagnosis of bacterial infection in the patient with cancer, fever and neutropenia is difficult due to the poor inflammatory response. Several evaluations of acute phase reactants such as C-reactive protein (CRP) have been performed with diverse results. The aim of this study was to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and likelihood ratios (LR) for CRP in the diagnosis of bacterial infection of patients with cancer, neutropenia and fever.

Methods. We carried out a diagnostic test study. Pediatric patients with cancer and neutropenia (<500 AN/mm3) were selected. CRP was determined by nephelometry. Episodes were classified into the following groups: group I: microbiologically documented infection; group II: clinically documented infection; group III: fever of unknown origin; group IV: patients with neutropenia without fever. Sensitivity, specificity, PPV, NPV, receiving operating curves (ROC) and LR were calculated. Mann-Whitney U test and Kruskal-Wallis test were used for comparison of quantitative variables. For qualitative variables, χ2 test was used.

Results. There were 127 episodes distributed as follows: 29, 47, 20 and 31 for groups I, II, III and IV, respectively. Median CRP values were 282 mg/L for group I, 205 mg/L group II, 27.3 mg/L group III and 5.1 mg/L group IV (p <0.001). With a CRP value of 60 mg/L, we obtained a sensitivity of 94%, specificity 94%, PPV 6% and NPV 92%. LR for a positive test was 15.6 and LR for a negative test was 0.06.

Conclusions. CRP is a useful and economically feasible test for diagnosis of bacterial infection in patients with cancer, neutropenia and fever.

Key words: C-reactive protein, cancer, neutropenia, bacterial infection, fever.

INTRODUCTION

In cancer patients, fever is a common sign. In 60–70% of cases there is an infectious origin. Other causes of fever include disease relapse or progression, adverse effects of certain drugs, transfusion reaction or adrenal crisis.

1 Servicio de Infectología,
2 Unidad de Investigación en Epidemiología Clínica,
3 Laboratorio de Inmunología,
4 Servicio de Oncología,
5 Servicio de Hematología, Unidad Médica de Alta Especialidad, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México

Correspondence: Dr. Fortino Solórzano Santos
Servicio de Infectología
Unidad Médica de Alta Especialidad
Hospital de Pediatría
Centro Médico Nacional Siglo XXI
Instituto Mexicano del Seguro Social
México, D.F., México
E-mail: fortino.solorzano@imss.gob.mx

Received for publication: 5-24-12
Accepted for publication: 9-5-12
76.6% with a CRP value of 40 mg/L in 75 children with cancer.\textsuperscript{10} In another study of adult patients, using the same cutoff value, sensitivity was 100% although the specificity was very low (7%). Cut-off values in these studies were arbitrarily selected.\textsuperscript{11}

There are studies that report that this test has little use. With a cut-off level of 50 mg/L, Riikonen et al. observed a specificity of 100%, with a sensitivity of 24%.\textsuperscript{12} Katz et al., when evaluating levels between 20 and 100 mg/L, observed a sensitivity of 22-71% and specificity of 32-71%, suggesting that the variations were related to the isolated microorganism.\textsuperscript{13} Recently, some authors have used the test to guide antimicrobial treatment.\textsuperscript{14,15} However, the number of patients included in these studies do not allow for generalizations of its application to all centers that care for children with cancer.

The aim of this study was to evaluate the usefulness of the quantification of CRP in children with cancer, neutropenia and fever by calculating the sensitivity, specificity, positive and negative predictive values and likelihood ratios, as well as to obtain the best value for differentiating between patients with microbiologically or clinically documented infection and children with fever of noninfectious origin.

**Patients and Methods**

The study was conducted in the Hospital de Pediatria (HP) of the Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (CMNSXXI, IMSS), which is a tertiary care center. We included children with leukemia, lymphoma or solid tumors admitted with fever and severe neutropenia during a 2-year period. For the comparison group, patients with neutropenia without fever were included. We excluded patients receiving antimicrobial therapy 1 week prior to the episode of neutropenia and fever as well as patients subjected to surgical procedures 5 days prior to fever onset and patients with third-degree malnutrition, or with liver disease or liver failure. Patients whose CRP sampling was not performed at the time of blood collection for blood cultures were also eliminated. Blood cultures were processed according to international standards (American Society of Microbiology).\textsuperscript{16}

Fever was defined as a single temperature of ≥38.3°C or axillary or oral temperature of 38°C for =4 h. Severe neutropenia was defined as AN count ≥500/mm\textsuperscript{3}. Patients with fever had a complete medical history and physical examination performed. At least two peripheral blood cultures were obtained, and an additional one via a central venous catheter if present. At the same time, 1.0 mL of blood was obtained for CRP determination. After obtaining cultures, an empirical antimicrobial scheme was begun with ceftazidime (150–200 mg/kg/day) and amikacin (20 mg/kg/day). Modifications to the antimicrobial scheme were made by the treating physicians who were unaware of the CRP levels for each patient. Patients were evaluated on a daily basis until fever and neutropenia were in remission.

A 0.5-mL plasma sample was required for quantification of CRP. Formation of the complex comprised of specific antibodies and CRP was detected by light refractometry using nephelometry (Nephelometer 100-Analyzer, Behring Co., Marburg, Germany). The quantity was determined according to mg/L. For reliability of measurements, sample aliquots were stored at -70°C until use and PCR quantification was done in duplicate. A correlation of $r = 0.96$ (Spearman rho) was obtained in the paired measurements. Additionally, with each series Reuma IV/T, SL/1 and 2 control samples were measured to control the precision and accuracy of the tests. For quantification of CRP, a 0.5-mL sample of plasma was required. Audits were also conducted by external laboratories. The procedure was performed by an investigator who was unaware of the condition and diagnosis of patients. Patients were divided into four groups according to their clinical condition:

- **Group I–Documented microbiological infection**
  - Bacterial or fungal growth in cultures of sterile sites with or without infectious focus identified.

- **Group II–Clinically documented infection**
  - Patient with clinical data of infection without isolation of the causative organism.

- **Group III–Fever not associated with infection**
  - Episode of fever without clinical or microbiological evidence of infection, with symptom remission without association with the beginning of the antibiotic scheme or when it was determined that fever was attributed to neoplastic activity.

- **Group IV–Patients without fever**
  - Cancer patients who, at the time of evaluation, had severe neutropenia without fever or clinical evidence of an infectious process. This group constituted the control group.
Sample Size
A value of \( p < 0.05 \) was calculated with an estimated sensitivity and specificity of 80% \([p = 0.80, 1 - p = 0.20]\) and total amplitude of the interval of proportion of ±0.20, with which a minimum size sample of 124 patients was estimated.

Ethical Aspects
The study was classified as minimum risk and approved by the Local Research and Ethics Committee of the HP, CMNSXII. Verbal consent was requested from parents or patient guardians for CRP sampling.

Statistical Analysis
Nonparametric statistics were used for non-normally distributed quantitative variables. Measures of central tendency and dispersion, respectively, were used for median (Md) and interquartile limits (Liq = 25-75 percentiles). Mann-Whitney U test and Kruskal-Wallis test were used for comparison of quantitative variables between two or more groups, respectively. \( \chi^2 \) or Fisher’s exact test was used to compare qualitative variables. To evaluate the correlation between CRP measurements that were processed in duplicate, Spearman rho test was performed. For these tests, \( p \leq 0.05 \) was considered significant.

To determine the usefulness of CRP, a Bayes analysis was performed. Patients in groups I and II were considered true positives and groups III and IV as true negatives. The optimum values of CRP to obtain the best levels of sensitivity and specificity were established with receiver operating curves (ROC). Calculations were performed for positive predictive value (PPV) and negative predictive value (NPV) for each of the cut-off levels as well as for likelihood ratios and post-test probability. For sensitivity, specificity, PPV, and NPV, confidence intervals were calculated at 95% (95% CI). Statistical analysis was performed using SPSS v.18.0.

RESULTS
A total of 112 patients were included, 14 of whom were excluded, with 98 subjects remaining for the final analysis. Average age of the subjects was 9.3 ± 3.9 years. There were 57 males (58.2%) and 41 females (41.8%); 61.2% of patients had a diagnosis of leukemia, with acute lymphoblastic leukemia being the most common (Table 1). There were 42 patients in relapse of the underlying disease.

### Table 1. Comorbidities in study patients

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>49</td>
<td>50.0</td>
</tr>
<tr>
<td>AML</td>
<td>11</td>
<td>11.2</td>
</tr>
<tr>
<td>NHL</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>7</td>
<td>7.1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>7</td>
<td>7.1</td>
</tr>
<tr>
<td>Other sarcomas</td>
<td>4</td>
<td>4.1</td>
</tr>
<tr>
<td>HL</td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>100.0</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NHL, non-Hodgkin’s lymphoma; HL, Hodgkin’s lymphoma.

### Episodes
Of the 98 patients, we analyzed a total of 127 episodes, which were distributed among the four groups (Table 2). Of the total group, the median for the last dose of chemotherapy was 15 days (Liq 9-21) and the median for the days of fever before admission was 1 day (Liq 1-2). Group I consisted of 29 episodes. The most commonly isolated organisms were *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* (25.8, 16.1 and 13%, respectively). *Candida* was isolated in two episodes. In two episodes two different microorganisms were isolated.

Group II included 47 episodes. In 29 episodes, the focus of infection was found localized (IIa) and in 18 episodes the infection was not located (IIb). Of the diseases diagnosed in subgroup IIa there was a predominance of cellulitis \( n = 7, 16.6% \), neutropenic colon \( n = 6, 14.3% \), pneumonia \( n = 6, 14.3% \) and acute gastroenteritis \( n = 6, 14.3% \). The average time for fever remission was 2 days (Liq 1.5-4.5). In the group of patients without focus of infection (IIb), the average time for fever remission was also 2 days (Liq 2-3). One patient had fever remission on day 7 and in another patient on day 14 antimicrobial therapy was initiated. In group III there were included 20 episodes of fever and neutropenia unrelated to infection, and in seven (36.8%) it was reported that the cause of the fever was secondary to disease relapse. The average time for fever remission was 1 day (Liq 1-5) and there was no relationship with the onset of empiric antibiotic treatment. In Group IV there were 31 episodes included. At the time of evaluation, the majority were receiving chemotherapy for cancer.
**Table 2. Characteristics of patients according to groups of neutropenic episodes**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated</td>
<td>With focus</td>
<td>Without focus</td>
<td>Fever due to other causes</td>
<td>Neutropenia without fever</td>
</tr>
<tr>
<td>n</td>
<td>29</td>
<td>29</td>
<td>18</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>17 (58%)</td>
<td>16 (55%)</td>
<td>8 (42%)</td>
<td>9 (47%)</td>
<td>11 (35%)</td>
</tr>
<tr>
<td>Cancer stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Rejected</td>
<td>15 (51%)</td>
<td>18 (62%)</td>
<td>7 (37%)</td>
<td>7 (37%)</td>
<td>11 (35.5)</td>
</tr>
<tr>
<td>Final CT (days)</td>
<td>13</td>
<td>12</td>
<td>14.5</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Fever at admission (days)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (0.5-5)</td>
<td>—</td>
</tr>
<tr>
<td>Remission of fever (days)</td>
<td>4 (2-7.5)</td>
<td>2 (1.5-4.5)</td>
<td>2 (2-3)</td>
<td>1 (1-5)</td>
<td>—</td>
</tr>
<tr>
<td>AN (x10^9/L)</td>
<td>7</td>
<td>24</td>
<td>38</td>
<td>162</td>
<td>301</td>
</tr>
<tr>
<td>Days of antibiotic treatment</td>
<td>14</td>
<td>10</td>
<td>7.5</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>282</td>
<td>198</td>
<td>178</td>
<td>27.3</td>
<td>5.1</td>
</tr>
<tr>
<td>LDH (μg/L)</td>
<td>359</td>
<td>325.5</td>
<td>253</td>
<td>358</td>
<td>234</td>
</tr>
</tbody>
</table>

*Median (interquartile limits).
**Kruskal-Wallis χ².
ALL, acute lymphoblastic leukemia; CRP, C-reactive protein; CT, chemotherapy; AN, absolute neutrophil; LDH, lactate dehydrogenase.

**CRP Values**

The average CRP value for group I was 282 mg/L (Liq 174.3-385.5); for the subgroup IIa it was 198 (Liq 127.4-267). For subgroup IIb it was 178 (Liq 119-211) and for group III it was 27.3 mg/L (Liq 12.3-55.2). For group IV it was 5.1 mg/L (2.4-13.3 Liq) (Table 2). When comparing CRP values between groups I and II, we found no significant differences (p = 0.06), the same for subgroups IIa and IIb (p = 0.23). Therefore, it is considered as one group [patients with infection [I, IIa and IIb]]. The group of patients without infection constituted groups III and IV.

Average CRP values in the group of patients with infection was 201 mg/L (Liq 136.5-300.3) and 12.0 (2.8-29.5 Liq) for the group of patients without infection (p <0.0001).

When comparing CRP levels with the isolated organism in group I patients, it was observed that the averages of the test were higher for *S. aureus* (371 mg/L, Liq 220.5-446.2), *E. coli* (304 mg/L, Liq 143.3-394.5) and *E. faecalis* (294 mg/L, Liq 207-381), although no significant difference was observed (p = 0.35) (Table 3).

**Analysis of the Diagnostic Test**

In accordance with the ROC curves for the identification of bacterial infection, the optimal cut-off level of CRP was 60 mg/L. At this point, sensitivity was 94% (95% CI 89-99%), specificity was 94% (95% CI 88-100%), PPV 96% (95% CI 92-100%) and NPV 92% (95% CI 85-99%).

Using a CRP level of 60 mg/L, when comparing the group of patients with infection vs. group III and comparing the infected group vs. group IV, sensitivity, specificity, PPV and NPV values remained high.

When the usefulness of CRP with a value of 60 mg/L in patients with leukemia and solid tumors was evaluated, sensitivity, specificity, PPV and NPV values were higher (Table 4). The likelihood ratio of the different cut-off levels is shown in Table 5. With a CRP level >60 mg/L, the
Usefulness of C-reactive protein in the diagnosis of bacterial infection in the pediatric patient with cancer, fever and neutropenia

The likelihood ratio was 15.6 and 0.06 for a level below this value. It is noted that with a result of CRP <30 mg/L, no patient had infection, whereas with a number >100 mg/L, all patients were infected.

**DISCUSSION**

The origin of fever in neutropenic patients is a diagnostic and therapeutic problem. Depending on the series consulted, ~70% of patients in these conditions present with infection. Among the remaining patients, fever may be associated with transfusions, administration of chemotherapeutic agents, antimicrobials or tumor necrosis. 1, 2 Fever may be the only sign of serious infection given that the inflammatory response is minimal or nil. 4

Because of the high morbidity and mortality associated with infections in patients with severe neutropenia, the administration of broad-spectrum antimicrobial schemes is recommended. However, its use can lead to the induction of microbial resistance, fungal superinfections, side effects and increased treatment costs. 18

Due to the low identification of the etiologic agent of infection by routine testing for microbiological diagnosis (cultures, serology, antigen detection) in these patients, other diagnostic tests are necessary such as biomarkers that support the clinical diagnosis and meet the characteristics of speed, low cost, high sensitivity and specificity, without being affected by the underlying disease, transfusions or chemotherapy. 19, 20

CRP is perhaps the most evaluated biomarker used in different studies. However, in a recent meta-analysis 20 it was believed that its use should not be recommended as there is heterogeneity and inconsistency in all studies reviewed as well as the fact that results to discriminate between patients with and without infection are not conclusive.

In this study of evaluation of phase V diagnostic testing, there were 98 patients included with identical baseline clinical characteristics among groups, without identifying the cause of the fever at the time of their evaluation. Groups I and II were considered to be patients with infection. When jointly analyzing groups III and IV as patients without infection, sensitivity and specificity.

### Table 3. CRP values (mg/L) in accordance with isolated microorganism

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>n</th>
<th>CRP*</th>
<th>CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>371</td>
<td>220.5-446.2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5</td>
<td>304</td>
<td>143.3-394.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4</td>
<td>232</td>
<td>143.7-297.5</td>
</tr>
<tr>
<td>Coagulase-negative Staphylococcus</td>
<td>3</td>
<td>122.2</td>
<td>103-293.3</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3</td>
<td>294</td>
<td>207-381</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>210</td>
<td>138-282</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>1</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter lwoffii</td>
<td>1</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1</td>
<td>409</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>1</td>
<td>205</td>
<td></td>
</tr>
</tbody>
</table>

*Median.
†IL (interquartile limits).

### Table 4. Diagnostic test values in accordance with type of hematological disease

<table>
<thead>
<tr>
<th></th>
<th>Infected vs. noninfected</th>
<th>Solid tumors</th>
<th>Leukemia (95% CI)*</th>
<th>Solid tumors (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94% (91–100%)</td>
<td>96% (89–100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>89% (77–100%)</td>
<td>100% (96–100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>94% (88–100%)</td>
<td>100% (96–100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>89% (77–100%)</td>
<td>95% (86–100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Comparison between groups I and II vs. III and IV.
PPV, positive predictive value; NPV, negative predictive value.

### Table 5. Likelihood ratios (LR) for each cut-off level of CRP

<table>
<thead>
<tr>
<th>Cut-off level (mg/L)</th>
<th>LR for a positive result</th>
<th>LR for a negative result</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>4.9</td>
<td>0.02</td>
</tr>
<tr>
<td>50</td>
<td>6.0</td>
<td>0.04</td>
</tr>
<tr>
<td>60</td>
<td>15.6</td>
<td>0.06</td>
</tr>
<tr>
<td>70</td>
<td>15.5</td>
<td>0.07</td>
</tr>
<tr>
<td>80</td>
<td>15.5</td>
<td>0.07</td>
</tr>
<tr>
<td>90</td>
<td>22.5</td>
<td>0.10</td>
</tr>
<tr>
<td>100</td>
<td>44.5</td>
<td>0.11</td>
</tr>
<tr>
<td>110</td>
<td>86.0</td>
<td>0.14</td>
</tr>
<tr>
<td>120</td>
<td>91.0</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Comparison between groups I and II vs. III and IV.
remained elevated with the values of the diagnostic testing. When using the cut-off level of 60 mg/L selected by ROC, sensitivity and specificity of the test was 94%. Predictive values were >90%, which improved when patients were classified according to the type of cancer. Due to the 79% prevalence of infection, different cut-off points of CRP levels were tested to determine post-test probabilities. At a cut-off level of 60 mg/L, the probability of having an infection in subjects with clinical suspicion and with positive results was 98% and probability of no infection was 2% for patients with negative CRP and low suspicion of infection. Additionally, it was found that at the same cut-off level of CRP, the likelihood ratio calculated that it is 15.6 times more probable to find a positive result in patients with infection. With a negative test result (<60 mg/L) there is less than one tenth probability (0.06) of it being present in a patient with infection. At a CRP level of <30 mg/L, none of the patients with neutropenia and fever, negative cultures and without focus of infection had an infection. Similarly, all patients with levels >100 mg/L had a bacterial infection. As has been reported in other studies, CRP values in subjects with infection were elevated despite the neutropenia. All group IV patients (neutropenia without fever) in general received some type of antineoplastic chemotherapy and their CRP values were low (Md 5.1, Liq 2.4-13.3).

Moreover, although CRP levels in the group of patients with fever associated with noninfectious causes (III) were higher than those in patients with neutropenia and without fever (IV), 95% confidence interval was not interpolated with that of infected patients (p <0.001). Therefore, it is possible that tumor activity or undetected viral infections discretely elevate levels of CRP but do not reach the values of patients with bacterial infection. No significant differences in CRP levels were found among groups according to definition of infection, whether clinically or microbiologically documented (Groups I and II) (p = 0.06).

Some authors have carried out evaluations in which CRP serves as a deciding factor in determining suspension or maintaining antimicrobial treatment. In newborns, Ehl et al. chose a CRP level of 10 mg/L. When the value was higher and sepsis was suspected, antibiotics were continued. If CRP was low, in the absence of systemic inflammatory response or clinical suspicion of sepsis, treatment was suspended. Outcomes were satisfactory and the frequency of relapses was low (3/176). NPV was 99% (95% CI 95.4-99.9%). However, the results are still considered to be preliminary. In neutropenic children with fever, Santolaya et al. carried out an evaluation with CRP at a cut-off level of 40 mg/L, supported on the basis of clinical characteristics. The rate of cases with treatment failures was also low. However, the study population was small. Aquino et al. catalogued neutropenic patients with fever as “high risk” of developing serious infections when they presented one of the following characteristics: recent diagnosis or relapse, ill appearance, NA <200/mm³ or prolonged neutropenia (>7 days). “Low risk” patients are usually in remission and their general status is favorable. In addition to their blood cultures being negative, they have tendency towards fever remission and evidence of bone marrow recovery. So far, there is no recommendation to use a defined CRP value to continue or discontinue antimicrobials in a cancer patient with fever and neutropenia. Given the overall evaluation of the diagnostic test and taking into consideration that the likelihood ratios that in previous studies were not calculated, we can conclude that the test was useful in pediatric patients with cancer who are treated in this tertiary-care hospital to distinguish between infected patients and subjects with fever associated with noninfectious causes.

REFERENCES

Usefulness of C-reactive protein in the diagnosis of bacterial infection in the pediatric patient with cancer, fever and neutropenia

Multimodal study of hand hygiene in a third-level pediatric hospital

Irma Zamudio-Lugo,1 Abigail Meza-Chávez,2 Yazmín Martínez-Sánchez,3 María Guadalupe Miranda-Novales,4 José Guadalupe Espinosa-Vital,1 Roberta Rodríguez-Sing1

ABSTRACT

Background. Despite the simplicity and cost-effectiveness of hand hygiene, compliance is less than 60% in healthcare workers. Training activities are rejected because they are considered too conventional. The aim of this study was to implement a multimodal hand hygiene strategy to assess compliance in health care workers.

Methods. A time-series study was performed including three observation periods during 3 years. Five components were implemented in parallel: changes in infrastructure, availability of supplies, training and education for health care workers, monitoring hand hygiene practices and feedback were recorded.

Results. During the first period (2009), the prevalence of hand washing was 53.84; less than 10% complied with the “five moments” of hand hygiene. For the second period in June 2010 (n = 204), the prevalence was 62.74; 13.23% complied with the five moments. In 2011 the prevalence was 51; 38.9% complied with the five moments (p <0.05).

Conclusions. After implementing a multimodal strategy, compliance increased significantly with a similar prevalence.

Key words: Hand hygiene, compliance, multimodal strategy, prevalence.

INTRODUCTION

Semmelweis was the first to identify the need for hand hygiene for healthcare personnel while observing infectious complications in women in labor who were treated by physicians after performing autopsies.1 Despite doubts and discreditation regarding his hypothesis, years later many studies showed that hand hygiene is the most effective measure for the prevention of nosocomial infections.

Nosocomial infections occupy a significant place in public health because of the impact on quality of life, morbidity and mortality of patients treated in all hospitals worldwide, especially in developing countries.2

Despite the simple and cost-effectiveness of the recommendation, the backing of healthcare professionals, in general, is only <60%. The arguments or justification for not carrying it out are numerous and include excessive workload, lack of input, poor infrastructure and adverse effects of antiseptics on the skin, among others.3,4

Training activities are rejected because as a strategy they were considered to be too conventional. However, some studies demonstrated a change in attitude towards hand hygiene with these activities5 in addition to the secondary economic benefits that this represents for healthcare institutions.6

Pittet et al. demonstrated that, through a process of intervention that included the training and use of a multimodal approach, we achieved a remarkable adherence to hand hygiene from healthcare professionals, along with a significant reduction in the infection rate.7

These facts were used as a supportive measure for the Guide for Hand Hygiene in Health Care issued by the World Health Organization (WHO) in 2009, considering its importance and proposing implementation. This Guide

1 División de Epidemiología Hospitalaria, Hospital de Pediatría, Unidad Médica de Alta Especialidad, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México
2 División de Epidemiología Hospitalaria, Hospital de Cardiología, Unidad de Investigación en Epidemiología Hospitalaria, Hospital de Pediatría, Unidad Médica de Alta Especialidad, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México
3 División de Epidemiología Hospitalaria, Hospital de Oncología, Unidad de Investigación en Epidemiología Hospitalaria, Hospital de Pediatría, Unidad Médica de Alta Especialidad, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México
4 Unidad de Investigación en Epidemiología Hospitalaria, Hospital de Pediatría, Unidad Médica de Alta Especialidad, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México

Correspondence: Irma Zamudio-Lugo
División de Epidemiología Hospitalaria, Unidad Médica de Alta Especialidad, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., México
E-mail: irmazamudio537@gmail.com; irma.zamudio@imss.gob.mx

Received for publication: 5-24-12
Accepted for publication: 9-5-12
highlights the importance of complying with the optimum characteristics for carrying out the strategy of a multimodal study.8,9

The objective of this study was to implement a multimodal strategy for hand hygiene to assess adherence to the technique according to the five moments of hand hygiene.

MATERIALS AND METHODS

We conducted a study involving a multimodal approach during three different periods: December 2009, June 2010 and January 2011. During all three periods we applied the same study of hand hygiene. The multimodal strategy consisted of the implementation of five parallel components. The components were as follows: 1) changes in infrastructure and availability of materials for hand washing and alcohol-gel hand sanitizer, 2) training and education for healthcare professionals, 3) monitoring of hand hygiene practices and feedback mechanisms, 4) reminders (posters) in the workplaces and 5) establishment of a safety culture through hand hygiene with the participation of healthcare professionals and hospital management teams.

According to the definitions of the WHO Guide and The Joint Commission,8,10 the following variables were considered for the study:

- **Adherence**: observable behavior that begins in a reflexive manner and primarily favors closeness or compliance with the five moments prior to a proper technique for hand hygiene.

- **Hand washing**: using water and liquid or bar soap for hand washing.

- **Correct technique for hand washing hand hygiene**: using liquid or bar antiseptic soap and water with vigorous friction for a minimum of 15 sec for both surfaces of the hands, in between the fingers, followed by rinsing with water and proper closure of the faucet (when the faucet is not automatic, one must use a paper towel).

- **Five moments for hand hygiene**: 1) prior to patient contact, 2) prior to a sterile procedure or antiseptic task at a critical site with a high risk of infection to the patient despite the use of gloves, 3) after contact with patient’s body fluids or secretions 4) after contact with the patient and 5) after contact with objects in the patient’s environment.

- **Hand sanitizer**: alcohol-based hand hygiene products and those that require no-rinse, with vigorous friction of both hand surfaces and in between the fingers, and applied until the product is completely dry.

For each individual observed, we recorded either hand washing or hand sanitizer and the procedure according to the five components (Figure 1). The first observation period (December 2009) was performed after intensive training in the five moments for hand hygiene, standard precautions and transmission type, as well as the correct technique of hand hygiene within the framework of measures for prevention during the 2009 pandemic H1N1 influenza with a coverage of >85% of the workers. The observations were made by the staff of Preventive Medicine and Epidemiology at the Pediatrics Hospital after standardization of observation criteria through a pilot test (kappa index = 0.89).

For the second period (June 2010) the strategy included the delivery of leaflets and brochures, about the five moments for hand hygiene and the proper technique, to all clinical department heads and division heads of all the surgical, medical and radiological specialties in the hospital to be widely distributed and discussed by the responsible healthcare personnel with the nominal recorded evidence of trained personnel for the service. Observations were conducted by staff of Preventive Medicine and Epidemiology of the Oncology Hospital in the same medical center.

The third test was conducted in January 2011 without any additional strategy to the ongoing campaign that is continuously provided to surgical personnel, which is strengthened by the assessment and correction of technical practice and the five moments of hand hygiene. Demonstrations were organized for hospital wards and shifts in which the exercise of hand washing was performed with staff and families, in front of a sink. Hospital authorities were informed of the approximate date the multimodal tests were to be undertaken and that for these studies, and for the latter, the observers would be staff members of Preventive Medicine and Epidemiology of the Cardiology Hospital of the same medical center. Hospital staff members of Oncology and Cardiology Hospitals were standardized with the same criteria as the Pediatrics Hospital prior to measurements of the observations. Statistical analysis was performed using simple frequencies, prevalence, and $\chi^2$ rate adjustment for between-group comparisons.
RESULTS

In the first study conducted during December 2009 there were two periods for the observations: at 8:00 am for the morning shift and 3:00 pm for the evening shift. The observed number of employees was 169 (Table 1). The majority were nurses and pediatric residents and laboratory specialties. The prevalence of hand washing was 53.85%. The group with the highest adherence was the nursing staff. However, when we assessed the correct technique, prevalence was observed in 23.08% of the workers who washed their hands (Table 2). Of these 21 workers, the evaluation found that only 15 carried out the five moments of hand hygiene (Table 3).

For the second observation period (June 2010), we included three shifts: 6:00 am for the shift change from the night crew with the morning one, 9:00 am for the morning crew and 3:00 pm for the afternoon crew. We observed 204 healthcare workers (Table 1). The prevalence of hand washing increased to 62.74% and the correct technique occurred in 39.84% (Table 2). With respect to the five moments, we observed that 53 of the total number of employees washed or disinfected their hands (Table 3).

Finally, the third multimodal study in January 2011 was conducted to measure the three shifts at the same times as the previous period. We observed 339 workers. The prevalence of hand hygiene was 51%, the correct technique occurred in 66.4%, with a prevalence of almost 60% of the subjects who met with the entire process correctly.

When comparing the three observation periods, there was an increase in hand washing of the permanent and resident medical personnel from 2010 to 2011. Compa-
Table 1. Prevalence of hand hygiene in healthcare workers during three observation periods

<table>
<thead>
<tr>
<th>Variables</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Rate</td>
</tr>
<tr>
<td>Physicians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>11.24</td>
<td>5</td>
</tr>
<tr>
<td>Medical residents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>27.81</td>
<td>26</td>
</tr>
<tr>
<td>Nurses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>37.28</td>
<td>44</td>
</tr>
<tr>
<td>Laboratory personnel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.92</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>17.75</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>169</td>
<td></td>
<td>91</td>
</tr>
<tr>
<td>Adjusted rate*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56.17</td>
<td></td>
<td>59.66</td>
</tr>
</tbody>
</table>

*Rate was adjusted to compare results of 2010 with those of 2009 and vice versa. For 2011, they were adjusted with those of 2010.

Table 2. Prevalence of healthcare workers who carried out hand hygiene using the correct technique

<table>
<thead>
<tr>
<th>Variables</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Rate</td>
</tr>
<tr>
<td>Physicians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.49</td>
<td>1</td>
</tr>
<tr>
<td>Medical residents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>28.57</td>
<td>4</td>
</tr>
<tr>
<td>Nurses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>48.35</td>
<td>14</td>
</tr>
<tr>
<td>Laboratory personnel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.40</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13.19</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>91</td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Adjusted rate*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.40</td>
<td></td>
<td>33.80</td>
</tr>
</tbody>
</table>

*Rate was adjusted to compare results from 2010 with those of 2009 and vice versa. For 2011, they were adjusted with those of 2010. *p = 0.03

Table 3. Prevalence of healthcare workers who applied the “five moments” of hand hygiene

<table>
<thead>
<tr>
<th>Variables</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Rate</td>
</tr>
<tr>
<td>Physicians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.76</td>
<td>1</td>
</tr>
<tr>
<td>Medical residents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>19.05</td>
<td>1</td>
</tr>
<tr>
<td>Nurses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>66.67</td>
<td>11</td>
</tr>
<tr>
<td>Laboratory personnel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.76</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.76</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Adjusted rate*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>77.52</td>
<td></td>
<td>46.93</td>
</tr>
</tbody>
</table>

*Rate was adjusted to compare results from 2010 with those of 2009 and vice versa. For 2011, they were adjusted with those of 2010.
ring the adjusted rates, there was a statistically significant difference for the prevalence in 2010 ($p = 0.03$) compared to 2009 and 2011. However, when comparing adherence to proper technique, there was a statistically significant increase only in 2011 ($p = 0.033$). Finally, and in relation to the five moments of hand washing, there was no statistically significant difference ($p = 0.29$) found, although the rate in 2010 was lower compared to 2009 and 2011. When the comparative analysis was performed, laboratory personnel were not included ($n = 27$ in 2011) because the two previous studies did not include personnel in this category.

The prevalence of hand hygiene was similar in all three testing periods. When technique and adherence were recorded, differences were observed (Figure 2). Although the latter was high in the first test period, a lower number of subjects washed their hands. In the last period we observed that both the technique and adherence increased.

**DISCUSSION**

As part of the activities of the Surveillance Unit of the Division of Hospital Epidemiology, studies on hand washing were conducted. Previously, three studies per year were conducted (one every 4 months). These studies recorded the hand washing technique but did not include other items necessary to identify the areas of opportunity. Training for healthcare workers is provided through the headquarters of Promotion and Prevention of Health and the Subcommittee on Control of Hospital Infections. In 2009 when the influenza pandemic occurred, it was decided to incorporate the multimodal strategy proposed by the WHO. During the pandemic, 85% of the hospital staff were trained in 2 months by the team from the Infectious Diseases and Epidemiology. Because of this, a significant change was anticipated in the adherence to hand hygiene. However, despite a 53% prevalence of hand hygiene, few used proper technique and fulfilled the five moments. Therefore, this study sought to improve the adherence to hand hygiene through other strategies coupled with scheduled training.

This study was performed under the guidelines developed by various working groups for the control of infection and patient safety. A comprehensive training process was implemented with adherence to the cognitive aspect of healthcare workers. The focus was geared towards the process of infection transmission. Measurements were later implemented for adherence using the term "my five moments for hand hygiene."

The studies showed that although there was an increase in the prevalence of hand washing and also the technique or the use of alcohol-gel hand sanitizer, it did not necessarily meet the five moments. In particular, the healthcare worker failed hand washing after contact with patient body fluids or secretions (point 3) and after contact with items in the patient’s environment (point 5). Apparently, the message accompanying the information "before" is incorporated more easily.

In 2011, a similar prevalence was found, but a larger number of workers washed their hands with the correct technique, and compliance also increased to meet the five moments. We noted how important changes occurred in adherence to hand hygiene, particularly in meeting the five moments for hand hygiene, without noticeable changes in the prevalence of hand hygiene and hand sanitation (Figure 2). The hospital staff is aware of hand hygiene studies that take place every year. Because this study was carried out by medical staff from another unit, the routine activity of the healthcare workers was not disrupted because they did not identify the person who was conducting the study as part of the Surveillance Unit of their hospital and did not realize they were being observed for the process of proper implementation of hand hygiene. This type of measure has been successfully used in other studies.
In a later interview, the healthcare staff of this hospital expressed that, once the person conducting the study was identified, some staff members were motivated to show an image of excellence and a better quality of care for the pediatric patients, thus reinforcing proper practice. It is unlikely that the results were affected because most were informed later about the testing.

Some limitations of the study were due to the staff turnover in the hospital. We did not include the same number of individuals in each category; therefore, an adjustment was required to make the final analysis. Another problem was the registration of meticulous hand sanitation with the use of alcohol gel. Although this gel was available in dispensers in 100% of the hospital areas observed, and its distribution was wide (Pasteur tables, toilets, nursing stations, nightstands next to the patient), the persons who were recording this noted that it was "underutilized." However, it was not precisely quantitated. This detail constitutes an area of opportunity for future assessments as well as to ensure that the healthcare worker recognizes this as an efficient alternative and uses it more frequently. Hand sanitizers reduce the time spent in hand washing and the costs of antiseptic soap and paper towels for drying, while reducing water consumption.\(^{13,14}\)

In a study published from a highly specialized IMSS hospital, a prevalence of hand hygiene superior to the current study (60.2% in 299 workers) was reported. In addition, the factors were reported for noncompliance, among which were lack of items in stock and excessive workload.\(^{15}\) It is essential to ensure the availability of hand hygiene products and to ensure appropriate nurse/patient and physician/patient ratios as recommended to prevent situations due to excessive workload. Previously, files from the Pediatric Hospital reported a prevalence of hand washing as 50 to 60%. In recent years, the technical evaluation of hand washing was combined with the following steps: rub hands together for >15 sec, use an abundant amount of lather and use proper drying technique. In addition, hospital staff were administered surveys once or twice yearly to test their knowledge on the prevention of nosocomial infections and, although they emphasized the importance of hand hygiene, it was not sufficient to increase compliance with a proper technique.

Although there are different strategies to improve adherence, absence of resources and products to meet the established process of hand hygiene increases the risk of improper performance due to the lack of supplies.\(^{16}\) The latter can be evaluated during the study along with the registration, indicating both the correct technique as well as the times when hand washing takes place. The goal is to generate a habit to the hospital staff that is incorporated into their daily activities and will increase patient safety.

REFERENCES

2. Declaración conjunta de apoyo de Ministros de Salud de México, Colombia, Centroamérica y el Caribe al primer reto de la Alianza Mundial por la seguridad del paciente "Una Atención Limpia es una Atención más Segura”. Firmada en México D.F. el 21 de septiembre del 2007. Available at: http://www.who.int/gpsc/es/


**ABSTRACT**

**Background.** Pediatric and adolescent gynecology is undergoing a developmental phase worldwide. Since 1994, the Hospital of Pediatrics of the Centro Medico Nacional Siglo XXI (National Medical Center XXI Century) has been providing gynecological care for the pediatric population by a multidisciplinary team at the Pediatric Clinic of Gynecology. The objective of this study is to describe the reasons for gynecological consultation between 1996 and 2011.

**Methods.** We reviewed the consultation records of the Pediatric Clinic of Gynecology to identify patients’ ages at the time of care and diagnoses recorded during the study period.

**Results.** During the 15-year period, 3,200 consultations were given, averaging 226 consultations per year: 90% of these consultations were for patients with chronic disease and 10% for patients referred from secondary medical care hospitals but without underlying disease. The primary complaint was menstrual disorders (58%) followed by vulvovaginal pathology (16.1%), uterine malformations and tumors of the ovary and uterus.

**Conclusions.** The Pediatric Clinic of Gynecology has improved the quality of care for young girls and adolescents, particularly those with an underlying disease. It is important to detect patients with gynecologic problems from a primary care setting.

**Key words:** gynecology, menstrual disorders, uterine malformations, pediatrics, adolescents.

**INTRODUCTION**

Pediatric and adolescent gynecology (or infant-juvenile gynecology) is a relatively new specialty that is under development worldwide, especially in Latin American countries such as Argentina, Chile, Cuba, Colombia and Mexico. The history of infant-juvenile gynecology has its origin in 1790 when it was referred to as a “medical need...” But it was not until the mid-nineteenth century when the current concept of adolescent medicine came into play. One of the first documented adolescent healthcare services was in 1884 at a boarding school, while a special service for adolescents was established in the early twentieth century at Stanford University in the U.S. Later, in 1951, a unit for adolescents was created in Boston. This was the first location that began formal clinical training on the subject. Currently, in the U.S., adolescent medicine has been approved as a subspecialty.

The first adolescence service in Latin America was created by Dr. Dulanto Gutierrez in Mexico City who was a pioneer in promoting the training of professionals in this area, with an integrated and humanistic focus. Similar services were progressively added in other Latin American countries where the common denominator was the interdisciplinary perspective as the model for the care for adolescents.

The first physicians who recognized the need and importance for specialized care for female children and adolescents were European obstetrician-gynecologists. In 1940 the first gynecological service for children and adolescents was established in Czechoslovakia and one of...
the first papers on the subject was published, describing that this discipline comes from two medical specialties: pediatrics (adolescent care) and gynecology (infant-juvenile gynecology).4

In 1986, the American Society of Pediatric and Adolescent Gynecology was formed, whose main activity has been the dissemination of knowledge in the area, thus creating the Journal of Pediatric and Adolescent Gynecology, with Dr. Sanfilippo being one of the leaders. In Latin America, over the years, the International Federation of Infant-Juvenile Gynecology was founded, which focuses on physicians interested in pediatric gynecology and gives recognition to professionals trained in this area and who meet the corresponding requirements.2,4

The Hospital de Pediatria (HP) of the Centro Medico Nacional Siglo XXI (CMNSXXI) began providing gynecological care to the pediatric population beginning in 1994, but care such as multidisciplinary services was consolidated in 1996. Currently, this clinic consists of pediatricians trained in adolescent medicine as well as pediatric specialists in infant-juvenile gynecology, urology, endocrinology, infectious disease, radiology, genetics, and in psychiatry. Since then and to date, this clinic has provided care to patients of all pediatric ages with gynecological problems. The purpose of this study is to describe the reasons for consultation from 1996 to 2011 in the Pediatric Gynecology Clinic of HP, CMNSXXI.

PATIENTS AND METHODS

HP is a tertiary care medical unit comprised of multiple subspecialties. It is also a referral center for patients from different states of the Mexican Republic. It mainly serves pediatric patients with chronic and complex conditions. This has allowed the provision of care in the gynecology clinic to patients with a wide variety of conditions which, by its evolutionary course, affect the intricate balance of puberty and sexual maturity. Patients are referred to the gynecology clinic from general hospitals nearby with the motive of a consultation for a gynecological disorder or from a different pediatric subspecialty of the HP who refer patients with chronic (primarily cancer, renal, hematologic, neurologic or rheumatologic) problems with any gynecological complication, usually related to their underlying disease.

For the purpose of this study, we conducted a review of the records of requests to the Pediatric Gynecology Clinic for the identification of different diagnoses and the ages of the patients at the time of their care. Because of the great diversity of entities that involve Pediatric and Adolescent Gynecology, and for ease of the discussion, it was decided to group them into seven major categories:

I. Menstrual disorders: We included disorders from dysfunction of the hypothalamic-pituitary-ovarian (HPO) axis or thyroid dysfunction, as well as hyperandrogenic states, hyperprolactinemia, dysfunctional uterine bleeding, and abnormal and dysmenorrheic uterine bleeding.

II. Vulvovaginal pathologies: This group includes diseases such as vulvovaginitis (both bacterial and nonspecific), sexually transmitted infections, genital trauma, sexual abuse, congenital malformations, labial synchiae and skin diseases of various etiologies.

III. Disorders of pubertal development: This group includes precocious puberty and its variants, as well as delayed puberty including hypogonadism.

IV. Congenital malformations of the urogenital tract: These include uterus didelphys, bicornuate uterus, hemivagina, among others.

V. Breast pathology: Fibroadenomas, dermatosis.

VI. Uterine and ovarian tumors. Cysts, teratomas, adenocarcinoma, gestational trophoblastic disease and sarcoma botryoides.

VII. Sexuality and contraception. Visits for temporary contraception counseling or assessment of definitive methods such as tubal ligation and hysterectomy in patients with mental retardation.

RESULTS

During the period between 1996 and 2011, >3,200 consultations were provided with an average of 226 per year; 90% \((n = 2,800)\) of the consultations were for ambulatory patients referred by other HP specialists with a chronic disease, whereas the remaining 10% corresponded to patients without other underlying diseases that were referred from a secondary care clinic. During this period, the treated patients ranged from newborn to 17 years of age (Figure 1). When analyzing by age groups, the highest percentage corresponded to adolescents (70%), followed by the preschool-age group (10%).
Pediatric and adolescent gynecology in a tertiary level pediatric hospital: 15-year experience

Table 1 describes the proportion of consultations according to the gynecological problems. The chief complaint for the consultation was the care of the patients with menstrual disorders, in 58% of the cases, predominantly HPO axis dysfunction. The second reason for a consultation was vulvovaginal problems (16.1%), with vulvovaginitis being the most common, particularly in preschoolers. Also found among preschoolers and infants was labial synechiae, whether congenital or secondary, to untreated inflammatory or partially resolved problems, another frequent cause of consultation, along with other systemic diseases with dermatologic expression in the genital area such as lichen sclerosus, Behcet disease, ichthyosis or acrodermatitis enteropathica. Finally, it is noted that of the total consultations, breast disorders were the least observed.

Reported nosological diagnoses were mixed. Table 1 also shows the most frequent specific diagnosis, according to the proposed groups.

**DISCUSSION**

According to the results described, the experience in the clinic for children and adolescents in the HP Gynecology Clinic has been very rewarding. Each of the aspects of this discipline has been accomplished. During 15 years of continued learning—from the various challenges faced in caring for these patients—it has been possible that, currently, all of the cases have been favorably resolved. Of course, the time for each resolution depended on the individual patient.

Most of the patients who were treated had an underlying disease; therefore, the common problems observed and detected at a first level of care, such as contraception, physiological leukorrhea or vulvar trauma, among others, are underrepresented. This is because this hospital is a pediatric referral center where patients are received from first- and second-level care units after having received some form of treatment that did not resolve the problem or the care was inappropriate. Hence, the frequency of the various problems described in this paper is different from other publications.1

Generally, the most common problem in adolescent gynecology has been described as menstrual disturbances, which was also found in this review.2 Menstrual disorders are very common during the first 2 years after menarche; ~50% of adolescents have anovulatory cycles, and dysfunctional uterine bleeding is the most common manifestation.5,6 In our clinic, the main menstrual alterations are related to systemic chronic diseases, particularly with a hemato-oncological disease. In this group of adolescents, the main reason for the visit was hormonal menstrual suppression with the risk of major bleeding due to severe thrombocytopenia. The types of hematological disorders presented in these patients who have merited intervention because of hyperpolymeronorhea are acquired aplastic anemia, Von Willebrand disease, Glanzmann’s thrombasthenia or leukemia. In these patients, administration of drugs for menstrual inhibition has been successful in cases of

---

Table 1. Most frequent diagnoses according to the group of gynecological problems of patients treated at the Pediatric Gynecology Clinic, Pediatric Hospital, Centro Médico Nacional Siglo XXI

<table>
<thead>
<tr>
<th>Group of diseases</th>
<th>%</th>
<th>Most frequent nosological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual alterations</td>
<td>57.2</td>
<td>Ovarian dysfunction</td>
</tr>
<tr>
<td>Vulvovaginal pathology</td>
<td>16.1</td>
<td>Vulvovaginitis and labial synechiae</td>
</tr>
<tr>
<td>Ovarian tumor pathology</td>
<td>8.0</td>
<td>Benign ovarian tumors</td>
</tr>
<tr>
<td>Alterations in pubertal development</td>
<td>7.5</td>
<td>Delayed puberty</td>
</tr>
<tr>
<td>Sexuality and contraception</td>
<td>6.2</td>
<td>Consultation for contraception</td>
</tr>
<tr>
<td>Congenital urinary tract malformations</td>
<td>3.3</td>
<td>Uterine malformations</td>
</tr>
<tr>
<td>Breast pathology</td>
<td>1.7</td>
<td>Breast fibroadenoma</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
moderate or severe anemia to prevent a hemodynamic decompensation and reduce transfusion requirements.\(^5\)

Within this group, a lower frequency of problems of secondary amenorrhea has been observed as a manifestation of hypogonadotropic hypogonadism by ovarian toxicity associated with chemotherapy (mainly by alkylating agents) or subdiaphragmatic radiotherapy.\(^7\)

Another group of patients who frequently present menstrual disturbances are those patients with chronic kidney disease, which accounted for \(\sim 14\%\) of the total patients in our practice. In these patients, there is a wide clinical spectrum from secondary hyperpolymerorhea to uremia and HPO axis dysfunction, to oligo-amenorrhea due to hyperprolactinemia or thyroid dysfunction. In particular, monitoring of these patients has been prolonged, even after kidney transplantation because despite hormonal treatment, these patients continue with menstrual disorders. This has already been reported by other authors in the international literature.\(^5,9\) Also, menstrual disorders in patients with neurological diseases (such as sequelae of hypoxia-ischemia, central nervous system tumors, hydrocephalus, epilepsy, among others) have become gynecological complaints mainly because adolescents may present with opsomenorrhea, hyperpolymerorhea or catamenial epilepsy. In these patients, menstrual disorders are usually secondary to HPO axis dysfunction due to thyroid dysfunction, either due to polycystic ovarian syndrome or hyperprolactinemia associated with the use of anti-epileptic drugs (such as valproic acid) or antipsychotic agents.\(^10-12\)

According to our experience, the second most common reason for consultation was vulvovaginal problems. The most frequent of these problems was infectious or inflammatory vulvovaginitis, particularly among infants and preschool patients. During this age, anatomic and physiological factors determine increased susceptibility to infections because the vulva is an excellent medium for bacterial growth. During this age the skin is thin, there is no estrogenic stimulation and a neutral pH exists. However, most of the causes for vulvovaginitis are nonspecific. At these ages, poor hygiene is often observed, as well as fecal and urinary incontinence and the use of external irritants (moisture, chemicals, soaps or local baths). In contrast, in the sexually active adolescent population, the spectrum of the etiology of the condition changes after local vaginal estrogen stimulation, vulvovaginitis prevailing due to specific microbial agents (Neisseria gonorrhoeae and Chlamydia trachomatis) considered sexually transmitted diseases (STDs).\(^6,13\) Among vulvovaginal problems, we can highlight some rare cases of vulvovaginal condyloma in prepubertal patients. Patients who were detected with this condition during their gynecology visit had a mandatory comprehensive evaluation to rule out sexual abuse. Despite local treatment, this condition tends to be chronic with repercussions into adulthood with the risks of sexual problems and, in the case of pregnancy, vertical transmission to the fetus with the possibility of developing laryngeal papillomatosis.\(^14\)

A group of patients seen with relative frequency in pediatric hospitals are those with urinary tract and anorectal malformations. These patients are more likely to present Müllerian anomalies such as bicornuate uterus, uterus didelphys or hemivagina. Although the care of these patients represents only about 5\% of the cases, it is important to rule out these abnormalities in the prepubertal stage\(^15\) in order to avoid its diagnosis during puberty or in adulthood when the patients have severe dysmenorrhea, hematocolpos, hematometra, ectopic pregnancy or infertility, conditions that sometimes are preventable.\(^16\)

As mentioned, the lowest frequency for consultation in the Gynecology Clinic has shown to be in relation to breast pathology because it represented only 1.7\% of patients treated. In 99\% of these patients \((n = 54)\), the result was a benign mass. Fibroadenoma was the most common diagnosis followed by fibrocystic breast disease. This has also been observed in other hospitals similar to ours.\(^17\) In this context one should consider that although breast pathologies in children seem rare and benign, an examination should always be performed during the medical visit and self-examination should be recommended.\(^18\)

In regard to patients with tumor pathology, ovarian tumors have been detected, mainly as simple benign cysts, cystadenomas and mature teratomas. Most have been identified as part of the scrutiny of menstrual disorders where ultrasonography has played an important role. Due to the possibility that malignant lesions such as adeno-carcinoma may be present, all adolescent females are referred to the surgical oncology specialists for surgical management, as recommend by other authors.\(^19\) On the other hand, there have been isolated diagnosed cases of uterine rhabdomyosarcoma, botryoides tumor confined
to the vagina and gestational trophoblastic disease in the group of patients with tumors in whom the main manifestation was abnormal vaginal bleeding.20

Finally, a key aspect in the care of adolescents is counseling on sexual and reproductive health. In the experience of this Gynecology Clinic, this activity is a priority. However, for the type of patients we serve, generally the recommended contraceptive methods we have offered are preferentially to patients with chronic diseases. An adolescent group in particular is the kidney transplant patient because in the early posttransplant period, birth control is needed for graft preservation. Therefore, we have implemented discussions and personalized advice. Similarly, another group of patients who require special care are adolescents with progressive neurological disorders (such as difficult to control epilepsy, mitochondrial diseases or malformations of the central nervous system) in which, at the request of their parents, procedures of permanent contraception have been performed such as tubal ligation or hysterectomy.21-23

In conclusion, results of this study provide an overview of the varied and complex problems that may represent the spectrum of care for gynecological disorders during any pediatric age. We hope that this publication will contribute to the knowledge for the primary care physician because either the general practitioner or the pediatrician will identify, study and channel these issues in a timely manner for patients with any of the reported alterations, with the objective being to improve their care.

REFERENCES

Frequency and type of airway injury identified by bronchoscopic examination in newborns with prolonged endotracheal intubation in a neonatal intensive care unit

Heladia García,1 Hugo Ramírez-San Juan,2 Jorge Ramírez Figueroa,2 Raúl Villegas-Silva,1 Olivia Madrigal Muñiz1

ABSTRACT

Background. Endotracheal intubation and mechanical ventilation are frequently used resources in the Neonatal Intensive Care Unit. Higher morbidity has been observed as a result of complications. We undertook this study to report the frequency and type of airway injury in newborns with prolonged endotracheal intubation who underwent bronchoscopy examination.

Methods. Newborns (n = 150) who were intubated endotracheally for ≥5 consecutive days and who underwent bronchoscopy were included. We recorded the following variables: gestational age, birth weight, indications for intubation, size of endotracheal tube, number of reintubations, intubation length, indication for bronchoscopy, bronchoscopic findings, type of treatment for airway injury, and number of bronchoscopies.

Results. The main indication for bronchoscopy was atelectasis (persistent and/or recurrent); 96% of newborns had at least one injury. The most frequent were inflammatory type (67.3%), malacia (39.3%), and stenosis (28.7%). The most injured anatomic structures were the bronchi (31.6%), larynx (24%), and trachea (22%). For 126 patients, primary medical treatment was steroids. For 21 patients (14%), dilatation was performed under bronchoscopy; and for 7 (4.6%) patients, tracheostomy was performed.

Conclusions. The most frequent injuries were inflammatory-type. Persistent atelectasis was the principal clinical manifestation; therefore, it should be considered as an indication of bronchoscopic exploration to identify airway injury in newborns with prolonged endotracheal intubation.

Key Words: newborns, bronchoscopy, airway injury, atelectasis, tracheomalacia, bronchial stenosis, tracheostomy.

INTRODUCTION

Endotracheal intubation and mechanical ventilation are used routinely in the newborn intensive care unit (NICU) in newborns (NB) with severe breathing problems. Lives are often saved and survival of these children is favorably impacted. However, it was observed that morbidity rises significantly as complications can occur, such as airway injury (AI), which prevents early extubation, thus prolonging hospital stay with all the consequent problems.1-3

The anatomic characteristics of the airway of the newborn (NB) are different from those of older children. In NB the beginning of the larynx is at a level with the first cervical vertebra and is in contact with the soft palate and lumen of the larynx and the trachea is smaller. The subglottic region is the narrowest of all airway regions and, for this reason, any process that decreases its lumen >1 mm will cause a narrowing of the subglottic space in up to 60% of cases. Therefore, this area is more likely to present damage, given that the submucosa is comprised of loose areolar tissue, which favors the rapid development to edema.4,5

The frequency of AI in NB undergoing mechanical ventilation and who survive ranges between 44 and 47%. These patients develop subglottic stenosis in between 5
and 8% of cases. The most frequently reported injuries in neonates are edema, granulomas, acquired subglottic stenosis (this is the most common injury found to be associated in 90% of cases with prolonged endotracheal intubation), ulcers and tracheomalacia.2-23

The aim of the study was to identify the frequency and the main types of AI in NB with prolonged intubation and who underwent bronchoscopy.

PATIENTS AND METHODS

The study was conducted in the neonatal intensive care unit (NICU) and in the Pulmonary Disease Department of the Hospital de Pediatria (HP) of the Centro Medico Nacional Siglo XXI (CMNSXXI), IMSS, which is a tertiary-care referral hospital. NB, preterm and term, were included who had undergone endotracheal intubation ≥5 days and who underwent bronchoscopy. The study design was analytical cross-sectional.

The following data were recorded from the medical records: gestational age, birth weight, gender, age at admission, reason for intubation, intubation age, number of reintubations, time of intubation, extubation failure, reason for failure to extubate, weight at the time of bronchoscopy, indication for bronchoscopy, type of bronchoscopy (rigid or flexible), findings at bronchoscopy, type of treatment for AI, duration of treatment for AI and number of bronchoscopies.

Patients were identified from the database available from the endoscopy service and clinical files were later reviewed in the NICU or in the hospital’s medical records department. Data were recorded on a collection sheet specifically designed for the study.

All NB were orotracheally intubated at the time of bronchoscopy with an endotracheal transparent polyvinyl chloride without balloon tube. The tubes were fixed with adhesive tape. NB required mechanical ventilation. Types of ventilators used were cycled by time and limited pressure.

Bronchoscopy was performed in the NICU in the patient’s radiant warmer by two pediatric pulmonologists trained in bronchoscopy, with the assistance of the patient’s attending neonatologist. Electronic monitoring of heart rate, respiratory rate and oxygen saturation were carried out. Bronchoscopy was performed nasally through a face mask with a "T" adapter connected to the self-inflating resuscitation bag, after application of phenylephrine and local xylocaine and removal of the endotracheal tube. We used a Pentax FB-10X 3.5 mm fiberoptic bronchoscope with a working channel of 1.2 mm (Olympus Optical Co. Ltd., Tokyo, Japan) or Karl Storz rigid bronchoscope of 3.0 and 3.5 mm x 20, and Karl Storz Hopkins 2.7 mm telescope (Karl Storz Co., Tuttlingen, Germany). Children in whom we used the flexible bronchoscope were given midazolam for sedation (100-200 µg/kg/dose) and analgesia with buprenorphine (2-3 µg/kg/dose). In those patients on whom we used the rigid bronchoscope, we administered midazolam for sedation, muscle relaxant (vecuronium 50-100 µg/kg/dose) and analgesia with fentanyl (3-5 µg/kg/dose). To reduce the risk of bacteremia, antimicrobial prophylaxis with cefuroxime at 50 mg/kg/dose for three doses was administered (first dose was administered 30 min prior to the start of the procedure).

Statistical Analysis

Descriptive statistics were used for frequencies and percentages. The population was non-normally distributed so measures of central tendency and dispersion, medians and ranges were calculated. For comparison between groups, we used χ² or Fisher’s exact test.

RESULTS

Included in the study were 150 NB with prolonged endotracheal intubation who underwent bronchoscopy. Table 1 shows the demographic characteristics of the patients and the diameter of the endotracheal tube used, at least once, according to the patient's weight.

All patients were orotracheally intubated and with mechanical ventilation when the bronchoscopy was performed. The main indications for the procedure were atelectasis, stridor or dysphonia and failed extubations (Table 2). Of all patients, 96% had an airway abnormality. In 67 (44.7%) of patients, one AI was found, in 51 (34%) patients two AI were found, in 24 (16%) patients, three AI were found and in two (1.4%) patients there were four AI. In six (4%) patients the airway was found to be unchanged. Inflammatory lesions were the most frequent (67.3%) followed by malacia (39.3%) and stenosis (28.7%) (Table 3). For anatomic structure, 31.6% of the AI were bronchial, 29% laryngeal, 22% tracheal and 18.2% were mixed.

Medical treatment with systemice or inhaled steroids was administered to 126 patients with AI. We also used other
drugs such as inhaled albuterol, epinephrine, ipratropium bromide and phenylephrine. No treatment was given to 24 NB (Table 4). Treatment duration ranged from 1 to 10 days (median 5 days). Twenty-one NB with stenosis, in addition to medical management, underwent dilation. A balloon angioplasty catheter with a 2.5- to 3.5-mm balloon inflated to 2-4 atm for 30 sec was used. Of these patients, 18 were able to be extubated once the obstruction was resolved, i.e., immediately after the procedure. In seven patients (4.6%) a tracheostomy was necessary: four due to severe tracheomalacia, two due to stenosis and one due to tracheal stenosis.

Rigid bronchoscope was used in only six (4%) patients. In 74% of NB, bronchoscopy was performed, in 20% two
Frequency and type of airway injury identified by bronchoscopic examination in newborns with prolonged endotracheal intubation in a neonatal intensive care unit

When comparing children who had a weight ≤1500 g with those with a higher weight and who had an intubation time ≤14 days or more, there was no significant difference in the type of AI found (Tables 5 and 6). We also analyzed gestational, finding no differences.

**DISCUSSION**

Literature studies have reported that AI is multifactorial in origin and that endotracheal intubation time >5 days is the most important factor for its development. In these cases we suggest performing bronchoscopy in order to evaluate, detect and promptly treat airway alterations.4,6,8-12,20,24,25

The frequency of AI is very high compared with that reported by Da Silva and Stevens. These authors found mild subglottic stenosis and tracheal edema in one patient (of 227 NB <1500 g studied) and granulation tissue edema of the airway in three patients; however, they note that only four patients underwent bronchoscopy and in four there was damage found.9 Downing and Kilbride,10 in a group of 117 NB examined with bronchoscopy, reported 41% of patients with damage to the airway (tracheomalacia and subglottic stenosis). In the present study we reported all alterations found in the airway, including congenital malformations. However, if we consider only subglottic stenosis (4.6%) and tracheomalacia (12%), the figures are lower than those reported by Downing and Kilbride who reported 13% moderate or severe subglottic stenosis and 16% tracheomalacia. Furthermore, the characteristics of the patients and the hospital where the study was conducted are different. We included both preterm and term NB, and the hospital is a referral center where most patients are sent after several failed attempts at extubation and with suspicion of AI.

It has been mentioned that the inappropriate size of the endotracheal tube is a contributing factor to the AI, especially when it is larger than recommended for the child’s weight. Above all, it has been associated with subglottic stenosis. In this study, 25 patients weighing between 2000 and 4000 g who, in theory, should be intubated with a 3.5-mm internal diameter cannula, had a larger one placed at least one time. Although it is more likely that a tube with a larger caliber than recommended may cause injury, use of a smaller one can also cause mucosal lesion. With the movement of the child’s head, there is greater mobility of the tube, especially when a correct fixation of the tube has not been achieved.4,25-27 However, because the majority of the patients were initially managed in other hospitals, it was not possible to know the size of the endotracheal tube in all cases of intubation or all events of reintubation.

The main reasons found for reintubation were recurrent atelectasis, respiratory distress, stridor and apnea, which coincides with most authors.3,12,15,17,18 However, although data on how many children required reintubation and the reason for doing it was obtained, once extubated as sche-
duled, the number of accidental extubations was unknown. This is common in NICUs and one of the main factors contributing to AI because it causes frequent reintubations.

AI found most often were inflammatory similar to that reported by other authors. It is explained according to the type of tissue that exists in this anatomic area where edema easily develops.4,11,26

Frequency of subglottic stenosis reported in this study was lower than that reported in the literature.2,5,7,19,22 Treatment depends mainly on the type of injury; however, to date there is no well-established treatment regimen, rather there are several treatment options available. These include the use of systemic or inhaled steroids, although some authors believe that these measures are of little or only partial usefulness.

The patients studied showed good response to medical management, mainly consisting of steroids, achieving extubation after treatment in most of the cases. Healing of the AI was observed, which was confirmed in those who underwent a second bronchoscopy or by radiologic studies in cases of atelectasis.

Some authors mention that endoscopic procedures are the treatment of choice for acquired subglottic stenosis, before deciding on a tracheostomy.13,18 In this study, the majority of children with stenotic lesions (85.7%) who underwent dilation had a good response and were extubated successfully. Only three patients did not respond to dilation and underwent tracheostomy. The frequency of tracheostomy was very low and was only performed in seven patients and the indication was not exclusively prolonged intubation. Accordingly, it is proposed that the NB with prolonged intubation should be offered the most conservative management before carrying out tracheostomy.

The decision should not be based solely on intubation time because this procedure has greater risks. Mortality has been reported in between 6 and 24% of patients due to obstruction of the cannula or accidental decannulation.2,21,24,25 When comparing the weight, gestational age and intubation time there was no difference between those with lower weight, shorter gestational age and longer time of endotracheal intubation with respect to the type of AI presented. One would expect that smaller children and those with prolonged intubation would have a higher frequency of inflammatory lesions, stenosis and malacia because the anatomic structures of the airway in these children are smaller and more fragile.24,25 However, this was not demonstrated in the patient group studied. Some authors believe that because prolonged endotracheal intubation is the main determinant of AI. All patients with this condition should be routinely evaluated by bronchoscopy to remove the endotracheal tube in order to detect airway alterations early and, if necessary, provide timely treatment.11,17-19

Bronchoscopy, although an invasive procedure, is quite safe in experienced hands, with a low complication rate as shown in the patients studied. For this reason, children with clinical suspicion of AI, such as persistent or recurrent atelectasis, which was the most common clinical data in this group of children, or stridor or dysphonia as other authors found, should have airway exploration with bronchoscopy.11 On the other hand, it is also important to consider the evaluation of treatment because there is no standard protocol for AI management, especially inflammatory, which are the most frequent. Some studies have used both inhaled and systemic steroids and in other

### Table 6. Type of finding in accordance with time of endotracheal intubation

<table>
<thead>
<tr>
<th>Finding</th>
<th>Time of endotracheal intubation</th>
<th>Statistically significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤14 days (n = 9)</td>
<td>&gt;14 days (n = 141)</td>
</tr>
<tr>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>Inflammatory injury</td>
<td>4 44.4</td>
<td>97 68.8</td>
</tr>
<tr>
<td>Malacia</td>
<td>5 55.6</td>
<td>54 38.3</td>
</tr>
<tr>
<td>Stenosis</td>
<td>3 21.4</td>
<td>40 28.4</td>
</tr>
<tr>
<td>Ulcer</td>
<td>1 11.1</td>
<td>12 8.5</td>
</tr>
<tr>
<td>Granuloma</td>
<td>0 -</td>
<td>13 9.2</td>
</tr>
<tr>
<td>Others</td>
<td>1 11.1</td>
<td>22 15.6</td>
</tr>
<tr>
<td>Airway without changes</td>
<td>1 11.1</td>
<td>5 3.5</td>
</tr>
</tbody>
</table>

*Mantel-Haenszel χ² or Fisher’s exact test.*
Frequency and type of airway injury identified by bronchoscopic examination in newborns with prolonged endotracheal intubation in a neonatal intensive care unit

studies only mucolytics, β-agonists, etc. were used. We will have to evaluate these options, especially the use of systemic steroids, because of the potential harm posed to the neurological development of the NB.11,18,24,25,27-30

In this study the frequency of tracheostomy was low. It is considered that in identifying inflammatory processes of the airways by bronchoscopy, tracheostomy was avoided in children with prolonged intubation and allowed for management with local measures or with systemic anti-inflammatory agents. Furthermore, identification of alterations such as supernumerary or abnormal bronchi helped establish a prognosis and monitoring of these children. The benefits derived from performing bronchoscopy are unquestionable. The procedure was well tolerated and did not cause increased morbidity as reported by other authors. There was a low frequency of complications in our pediatric patients.12,14,31,32

Acknowledgments
We thank Dr. María Elena Furuya Meguro for collaboration in the review of the manuscript.

REFERENCES

Reliability of nursing records of anthropometric measurements of patients in a tertiary-care pediatric hospital

Miguel Ángel Villasis Keever,1 Norma Andrea Arias Villa,2 María Guadalupe Cedillo Rosas,2 Ivonne HERNÁNdez LUNA,2 Karla Cristina Emiliano ACEVES,2 Vianey Mora Gutiérrez,2 Martha Alicia Sánchez Ramírez,2 Jessi Nallely Zurita Cruz3

ABSTRACT

Background. In pediatric practice, obtaining vital signs and anthropometric measurements are essential elements for the diagnostic-therapeutic process of all patients. We undertook this study to determine the reliability of weight and height information recorded by the nursing staff of patients hospitalized in a pediatric hospital.

Methods. A group of nurses previously standardized in taking anthropometric measurements measured the weight and height of patients who were hospitalized during a 4-week period in 2011. Nursing records of weight and height at the time of hospital admission from each patient were also registered. These nursing records were compared with those obtained by the nurses standardized. Descriptive analysis was done and weight and height means were compared with a t-test.

Results. We included 192 patients from newborns to adolescents. Overall, the average weights and heights from nursing records were similar to those obtained by the group of nurses previously standardized in anthropometric measurements; however, we documented differences up to 12.6 kg in weight and up to 52 cm in height. In 20 patients there was no nursing record of height and in one patient there was no record of weight.

Conclusions. Height and weight records registered by the nursing staff can generally be considered reliable, but with some significant variations. Because of its importance, training and supervision interventions are needed to improve the quality of anthropometric measurements and to avoid errors.

Keywords: weight, height, nursing, quality of care.

INTRODUCTION

In pediatric practice, obtaining vital signs and anthropometric measurements are essential elements for the diagnostic and therapeutic process of any patient. As in other institutions or hospitals, in the Mexican Social Security Institute the determination of weight and height is an activity that the nursing staff routinely performs, regardless of the classification of the patient.

Anthropometric studies include weight and height measurements of the patient, making it the most reliable and specific indicator of nutrition. Weight and height (or length) are usually the most important anthropometric measurements. Length differs from height because it is measured when the individual is not standing.

As with any measurement, when measurements of weight and height are carried out, there may be three types of errors: primarily the instrument, the operator or observer and the individual in whom the measurements are made. In order to eliminate these errors, it is necessary to standardize the measurement procedure and to use the most appropriate tools, which must be operational under optimal conditions. In particular, within the standardization process, it is essential that the observer (in this case, the nursing staff) is trained to take weight and height measurements. There are various methods for taking these measurements including Zerfas, where the
Reliability of nursing records of anthropometric measurements of patients in a tertiary-care pediatric hospital

...main purpose is to ascertain that the measurements have the least amount of variability. There are authors who believe that the standardization process is effective when, in comparing data obtained from the measurements of an observer with a qualified person (gold standard), the differences are minimal. It is generally considered that the measurements of the height difference should be no more than 0.5 cm, whereas an acceptable weight variation can be up to 0.1 kg.6-10

Several studies have shown the importance of continuing education and training of the nurses for the reduction of occupational accidents and errors. The current trend in regard to the educational process responds to a constructivist theory where the nurses are critical and analytical in the professional exercise.11 There is evidence that the teaching-learning process facilitates the development of knowledge, skills and abilities in the nursing workload.12-15

In order to assess the quality of anthropometric measurements, since the 1980s several studies have been published that evaluated the variability of anthropometric measurements performed by healthcare personnel (including nursing staff), both for the development of research studies as well as in clinical practice.17-21 It has been demonstrated that, of the anthropometric measurements, weight and height are those with the least variability.17,18,22-24 However, there are challenges when carrying this out in newborns.21,25-27 Likewise, it is also described that the training process reduces the measurement variability. It is therefore recommended to carry out continuous assessments of the nursing staff for the purpose of maintaining the quality of anthropometric measurements.17-19,25,26 The objective of this study was to determine the reliability of the height and weight measurements registered by the nursing staff in hospitalized patients in a third-level pediatric hospital.

SUBJECTS AND METHODS

We performed an observational, cross-sectional, prospective and descriptive study in the Hospital de Pediatria, Centro Medico Nacional Siglo XXI. Prior to study initiation, the protocol was approved by the Local Committee on Health Research. This hospital has three floors for in-patient hospitalization where patients are divided according to age. Thus, on one floor there are patients <2 years of age where there are two ward rooms. Another floor has patients from 25 months to 7 years old with two ward rooms, whereas on the floor where patients 8- to 17-years of age are located, there are three ward rooms. In each ward there are 20–24 beds.

Six nurses evaluated the patients with standardized methods to perform anthropometric measurements of weight and height. Standardization was performed according to the proposal of Flores-Huerta et al.9 who verified the variability of intra- and interobserver measurements using analysis of variance for repeated measures in seven patients. Once minimal variability was established, the study was initiated and carried out during September 2011. We included patients who were admitted 24 h prior, to any of the seven wards. We excluded those with any physical limitation or medical conditions that would prevent measuring their weight and height or when parents or patients refused to participate. Weight and height were measured by two nurses for each of the selected patients. According to the age, weight was obtained with platform scales or infant scales and height with stadiometer or infantometer.9 These data were considered as “actual.” Subsequently, from the nursing record sheet, we registered the age, gender, and diagnosis upon admission for each patient, and the weight and height that was recorded at the time of admittance into the hospital ward.

Statistical Analysis

A descriptive analysis of each of the variables is presented in accordance with their scale of measurement; simple frequencies and percentages were used for qualitative variables, whereas quantitative variables are presented as mean and standard deviation. Due to the diversity of the patient’s ages, weight and height data were analyzed according to the hospitalization floor of the patient. Averages were compared with t test for independent samples. Statistical analyses were carried out using SPSS v.15.0 (Chicago, IL).

RESULTS

During the study period, a total of 359 patients were admitted to the hospital. Anthropometric measurements were able to be taken in 192 patients (50.5%). Table 1 presents the general characteristics of the children included, noting that there was a similar proportion of patients according to gender, and the largest percentage correspond to school-age and adolescent patients (40.1%). The main reason for
that the greatest variability occurred more frequently in patients who were hospitalized during the evening shift (Figure 3).

Moreover, the height data obtained is shown in Table 2 and Figure 4. As occurred with weight, averages of the records in the nursing sheet and actual data are virtually identical. However, 20 patients did not have their height recorded. When analyzing differences in the recorded height and the actual measurement according to hospital floor, it was found that the greatest variation was up to 3 cm in patients from 25 months to 7 years-old (third floor), 4 cm in infants (fourth floor) and 52 cm in patients >8 years of age (fifth floor). As with weight, this variation also occurred in one case per floor (Figure 5). According to percentages, upon comparing the variability of the measurements, it was observed that the greatest variation occurred in patients admitted during the morning shift (Figure 5).

DISCUSSION

Anthropometric measurements of weight and height in children is of paramount importance for the assessment, diagnosis and treatment of these patients, e.g., when calculating parenteral solutions and for medication dosing. 27,28 For these reasons, it is necessary for records in the nurses’ station to be reliable and based on the proper carrying out of the determination of weight and height.29,30

In regard to the results obtained in this study assessing measurement reliability of weight/length of 192 patients and nursing records for a period of ~4 weeks, it was found that the variability of the data recorded, in general, is minimal. However, there were some cases where it was determined that the recording of weight or height was significantly different according to the measurements of the previously standardized nurses. Approximately 10% of the cases did not have either weight or height recorded.

Table 1. General characteristics of the study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>97</td>
<td>50.5</td>
</tr>
<tr>
<td>Female</td>
<td>95</td>
<td>49.5</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants (&lt;24 months)</td>
<td>48</td>
<td>25.0</td>
</tr>
<tr>
<td>Preschool-age (25 months–7 years)</td>
<td>67</td>
<td>34.8</td>
</tr>
<tr>
<td>School-age and adolescents (8–17 years)</td>
<td>77</td>
<td>40.1</td>
</tr>
<tr>
<td>Principal disease or reason for admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncological disease</td>
<td>69</td>
<td>36</td>
</tr>
<tr>
<td>GI disturbances</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Surgical problems</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Kidney diseases</td>
<td>19</td>
<td>9.8</td>
</tr>
<tr>
<td>Cardiac diseases</td>
<td>18</td>
<td>9.3</td>
</tr>
<tr>
<td>Neurological diseases</td>
<td>15</td>
<td>7.8</td>
</tr>
<tr>
<td>Respiratory diseases</td>
<td>11</td>
<td>6.3</td>
</tr>
<tr>
<td>Endocrinological diseases</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>100</td>
</tr>
</tbody>
</table>

GI, gastrointestinal.

admission was an oncological disease (36%) followed by gastrointestinal (13%) or surgical (10%) problems.

In Table 2 and Figure 1, a comparison of the weight data obtained for each floor is shown. In general terms, both the averages obtained from the nursing records as well as those carried out by the group of nurses are almost identical. It is, however, worth mentioning that in one case there was no record of the weight on the nursing records. However, when analyzing the differences between weight recorded and actual weight, some variations were found. The greatest variation was 5.0 kg in a preschool-age patient, 2.950 g in an infant and 12.6 kg in a school-age patient (Figure 2). Despite this, there was no statistical difference between the nursing records and the actual weight. Comparing the variability of measurements of weight according to the staff–shift in which patients were treated, it was observed the greatest variability occurred more frequently in patients who were hospitalized during the evening shift (Figure 3).

Moreover, the height data obtained is shown in Table 2 and Figure 4. As occurred with weight, averages of the records in the nursing sheet and actual data are virtually identical. However, 20 patients did not have their height recorded. When analyzing differences in the recorded height and the actual measurement according to hospital floor, it was found that the greatest variation was up to 3 cm in patients from 25 months to 7 years-old (third floor), 4 cm in infants (fourth floor) and 52 cm in patients >8 years of age (fifth floor). As with weight, this variation also occurred in one case per floor (Figure 5). According to percentages, upon comparing the variability of the measurements, it was observed that the greatest variation occurred in patients admitted during the morning shift (Figure 5).

Table 2. Comparison of the weight and height measurements according to hospitalization floor

<table>
<thead>
<tr>
<th>Hospitalization floor</th>
<th>Recorded weight (kg)* Average (SD)</th>
<th>“Actual” weight (kg)** Average (SD)</th>
<th>Recorded height (cm)* Average (SD)</th>
<th>“Actual” height (cm)** Average (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third</td>
<td>17,159.6 (6,238.9)</td>
<td>17,100.0 (6,252.5)</td>
<td>103.0 (16.0)</td>
<td>103.1 (15.8)</td>
</tr>
<tr>
<td>Fourth</td>
<td>7,025.9 (3,517.4)</td>
<td>7,018.1 (3,506.9)</td>
<td>103.0 (16.0)</td>
<td>103.1 (15.8)</td>
</tr>
<tr>
<td>Fifth</td>
<td>42,777.2 (17,258.6)</td>
<td>43,121.2 (17,038.7)</td>
<td>142.8 (17.9)</td>
<td>143.7 (16.3)</td>
</tr>
</tbody>
</table>

*Data obtained from the nursing records at the time of admission.
**Data obtained from the measurements taken by the study authors.
Reliability of nursing records of anthropometric measurements of patients in a tertiary-care pediatric hospital

The most common variations observed were in regard to weight; however, it should be noted that these data may be different due to conditions unrelated to the nursing staff because these data are subject to change according to the patients’ conditions or to the measurement device. For the patient, weight is more susceptible to changes due to health conditions such as edema, dehydration, emesis or the time when it was measured.

In this sense, a limitation of our study was the difference in the time the measurements were taken by the trained nurses and the nursing records; in the nursing records the data were obtained at the time of hospitalization, whereas the actual weight and length were measured some hours later. This may explain the variations, particularly for weight. However, this situation does not seem reasonable for cases where there

---

**Figure 1.** Comparison of the nursing records of weight (weight 1) and “actual” weight (weight 2).

**Figure 2.** Variability of weight measurements.

**Figure 3.** Variability of weight measurements according to shift.
was a large variation between the recorded weight and the actual weight or height.

Another important point is that measurement variability may be related to the reliability of the instrument. Therefore, it is always necessary to check the proper functioning of the equipment each time a measurement is to be taken. In addition, these procedures must be performed in the best condition for the patient, in other words, with the lightest amount of clothing, without shoes, preferably before meals and with empty bladders.9

According to the results of this study, the following suggestions are made to improve the reliability of nursing records of weight and height:

- Have the necessary working devices for measuring weight and height according to the child’s age
- Check for properly functioning scales
- Measure the weight and height according to standardized guidelines
- Supervision by qualified personnel that the procedures are carried out in accordance with the appropriate techniques, consistent with the age of the pediatric patient
- Above all, provide ongoing training, both to the nursing staff as well as the medical staff, to provide the best quality of care

In conclusion, results of this study demonstrated that the nursing staff records for weight and height are generally reliable, although there are cases with significant variations. Therefore, continuous monitoring is needed to instruct how to perform these procedures to ensure quality care for pediatric patients.
REFERENCES
Steroid-resistant nephrotic syndrome: 15 years experience from the Hospital de Pediatría, Centro Médico Nacional Siglo XXI

María Alejandra Aguilar Kitsu, Claudia del Carmen Zepeda Martínez, María del Pilar Ibarra Cazares, Juana Lorena Sánchez Barbosa, Ramiro Alejandro Luna Sánchez, María Leticia Mendoza Guevara, Karina Diaz de León, José Manuel Ubillo

ABSTRACT

Background. In 1997 the Clinic for Nephrotic Syndrome was established at the Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (Mexico City); 30-50% of children with steroid-resistant nephrotic syndrome (SRNS) develop chronic renal failure and 60-80% achieve remission with cyclosporin. The objective of the study was to report treatment response and prognosis using the described scheme in a group of patients with SRNS.

Methods. Retrospective study in children with SRNS was done. Remission frequency and renal survival were measured.

Results. One-hundred fifty seven patients were studied; 66.7% were male. Mean age at diagnosis was 5.9 ± 4.2 years. Biopsies showed 33 results (21.9%) with minimal changes (MC), 74 (49%) with diffuse mesangial proliferation (DMP) and 44 (29.1%) with focal segmental glomerulosclerosis (FSGS). Mean follow-up time was 59.3 months (minimum 3 months, maximum 178 months); 59% were on cyclosporin and 17.3% cyclophosphamide. Twenty six patients received both treatments and six patients received all three medications; 78.2% entered remission, 5.8% were on dialysis and 1.9% died. Five-year renal survival was 92.9% and 10-year survival was 80%. Remission in patients with MC and DMP was 79.8% and 86.5%, respectively and in FSGS was 59.1%. Chronic renal failure was found more often in FSGS (20.4%). Risk of developing renal failure with FSGS compared with DMP was 4.7 times and FSGS compared with MC risk was 8.7 times greater.

Conclusions. Similar rates of remission and better renal survival were found compared with the literature.

Key words: steroid-resistant nephrotic syndrome, children, outcome.

INTRODUCTION

Idiopathic nephrotic syndrome (INS) in children is one of the most common glomerular diseases in the pediatric age. The response to steroids currently is the best prognostic factor in this disease. Less than 3% of the patients with steroid-sensitive nephrotic syndrome evolve to chronic renal insufficiency (CRI) compared with 50% of those patients who are resistant to steroids or steroid-resistant.1 In general, steroid-resistant nephrotic syndrome (SRNS) is defined as the lack of remission after 4 weeks of prednisone at 60 mg/m²/day. However, the French Society of Nephrologists adds "followed by three boluses of methylprednisolone (MPN)." Approximately 10% of children with NS are steroid-resistant.2

Treatment objective is to achieve remission of the NS and slow the progression of kidney damage. With this goal, various schemes have been used. In the study by Cattran et al., cyclosporin was considered as a first-line treatment in children.3 Other drugs used include bolus MPN,4 cyclophosphamide5 and mycophenolate mofetil (MMF).6 In 1997, the Clinic for Nephrotic Syndrome was established at the Hospital de Pediatría, Centro Médico Nacional Siglo XXI (HP CMNSXXI) and uses the same protocol. The objective of this study was to report the response to treatment and the prognosis with this scheme in these patients.
**PATIENTS AND METHODS**

We conducted a retrospective, longitudinal study, reviewing all the cases of INS who were evaluated at the Department of Nephrology, HP CMNSXX, from 1997 until March 2012. During this period, the patients were evaluated in the NS clinic by the same group of pediatric nephrologists. In each query renal function was evaluated (with serum creatinine levels) and the activity of NS (with albumin, cholesterol, triglycerides and proteinuria). Patients are discharged after 2 years of complete remission or once they reach the age of 17. Those patients with persistent proteinuria or renal function impairment continue with nephrology follow-ups in the predialysis clinic.

In cases where the first episode of NS was in a patient >8 years old, a renal biopsy is performed. If there are minimal changes (MC), a scheme of prednisone is administered. If it is diffuse mesangial proliferation (DMP) or glomerulosclerosis (GS), steroid treatment is initiated. Renal biopsy was performed on steroid-resistant patients. The usual treatment scheme in SRNS is as follows (Figure 1):

- Cyclosporin at 2-5 mg/kg/day divided into two doses was given as an initial treatment. If the patient presents with impaired renal function, cyclophosphamide was begun.
- In case of lack of response to cyclosporin (cyclosporin resistance) after 6 months of treatment or if there is elevated creatinine during treatment with cyclosporin, cyclophosphamide bolus will be initiated with 500 to 750 mg/m²/dose divided into 2 days. Boluses are administered each month and can last 6 to 12 months, according to the time that remission is achieved.
- In case of persistent active disease at 6 months after treatment with cyclophosphamide, it will be considered as unresponsive and the patient will then receive MMF at doses of 600 to 1,200 mg/m²/day.
- Regardless of the scheme used, with the initiation of any treatment to induce remission, prednisone at 1 mg/kg/day is additionally given. However, if the initial proteinuria is >100 mg/h/m², MPN boluses are administered at 10 mg/kg/day for 3 days. At 4 weeks, a gradual reduction of prednisone will begin.
- In the case of relapse, prednisone will be reinitiated at 1 mg/kg/day and a referral will be given for three MPN boluses at 10 mg/kg/day. If persistent, even in the nephrotic range after 2 months despite treatment with MPN, cyclosporin treatment will be changed to cyclophosphamide or from cyclophosphamide to MMF.

**Definitions**

SRNS was considered when any of these conditions were met: patients receiving a steroid scheme for <4 weeks at 60 mg/m²/day and persisting with proteinuria >40 mg/h/m² or patients in whom a renal biopsy was conducted because of being >8 years of age at the time of submission of the NS and whose histopathological result was DMP or focal segmental glomerulosclerosis (FSGS).

Complete remission was defined as proteinuria <4 mg/h/m² and serum albumin >3.5 g/dL. Partial remission was considered when proteinuria was 4 to <40 mg/h/m² or hypoalbuminemia between 2.5 and 3.5 g/dL. NS was considered active if there is proteinuria >40 mg/h/m² at the time of evaluation.

Deterioration of renal function was considered when creatinine concentrations were presented at >1.5 mg/
dL on three consecutive measurements. Stage 5 chronic renal disease (CRD) was defined as glomerulofiltration rate (GFR) measured by creatinine clearance of <15 mL/min/1.73 m² or entering into dialysis treatment. Renal survival was defined as the percentage of patients with no renal function impairment or do not enter dialysis therapy.

**Statistical Analysis**

Descriptive statistics were used (frequencies and percentages). All patients in the group with SRNS were classified according to the type of treatment received and initial histological imaging. For comparison according to treatment group, we used the main groups: only cyclosporin, only cyclophosphamide and combination of cyclophosphamide and cyclosporin. For the comparison of groups, we used ANOVA for quantitative variables and Kruskal-Wallis for comparison of percentages.

In the survival analysis, we used the Kaplan-Meier test, taking renal survival as censored events, patients who at the end of the study had renal function impairment or had entered peritoneal dialysis or hemodialysis. Odds ratios (ORs) and 95% confidence interval (CI) were used for risk quantification.

**RESULTS**

During the studied period, we reviewed the records of 268 patients with INS. Of these, 156 were considered steroid-resistant (58.2%); of these 66.7% were males. The mean age at diagnosis was 5.9 ± 4.2 years of age. Most underwent percutaneous renal biopsy (n = 151). The histopathology report corresponded to minimal changes in 33 patients (21.9%); diffuse mesangial proliferation (DMP) in 74 (49%) and FSGS in 44 (29.1%) (Table 1). Five patients did not receive a renal biopsy because they were under treatment with anticoagulants or because of a complication such as pancreatitis; 59% of the patients received only cyclosporin and 17.3% only cyclophosphamide. Twenty-six patients received both schemes and six patients received cyclosporin, cyclophosphamide and MMF sequentially. Five patients received only MMF or in combination with cyclosporin because they were initially treated in our hospital unit (Table 2). Average follow-up was 4.9 years (minimum 3 months and maximum of 14 years 10 months). Twenty patients were followed for >10 years.

### Table 1. Histological frequency of percutaneous renal biopsy in children with SRNS

<table>
<thead>
<tr>
<th>Histopathological image</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>33</td>
<td>21.9</td>
</tr>
<tr>
<td>Diffuse mesangial proliferation</td>
<td>74</td>
<td>49</td>
</tr>
<tr>
<td>FSGS</td>
<td>44</td>
<td>29.1</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>100</td>
</tr>
</tbody>
</table>

SRNS, steroid-resistant nephrotic syndrome; MC, minimal changes; DMP, diffuse mesangial proliferation; FSGS, focal segmental glomerulosclerosis.

### Table 2. Treatment schemes in children with SRNS

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin</td>
<td>92</td>
<td>59.0</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>27</td>
<td>17.3</td>
</tr>
<tr>
<td>MMF</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Cyclosporin-cyclophosphamide</td>
<td>26</td>
<td>16.7</td>
</tr>
<tr>
<td>Cyclosporin-MMF</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>Cyclosporin-cyclophosphamide-MMF</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>100</td>
</tr>
</tbody>
</table>

MMF, mycophenolate mofetil.

**Response to Treatment**

At the time of the last visit, 69.2% of the patients achieved complete remission, 9% partial remission (14) and 10.9% had active NS. Five patients had impaired renal function (3.2%), nine required renal replacement therapy (5.8%) and three patients died due to infections (1.9%) with no kidney damage (Table 3).

Five-year renal survival was 92.9% and 10-year renal survival was 80.4%. According to the treatment, renal survival was similar in the three groups with no statistically significant differences (Table 4).

**Comparison Using Histological Imaging**

Comparing age at the time of diagnosis according to the histological image was not statistically significant (p = 0.672). Considering the histological type, in relation to the age showed the following results: with MC it was 5.4 ± 4.8 years, with DMP it was 5.8 ± 3.9 years, and with FSGS it was 6.3 ± 4.4 years.

Total as well as partial remission was similar between patients with MC (79.8%) and those with DMP (86.5%). However, the percentage of patients in remission was lower in patients with FSGS, with 59.1% (p = 0.019).

Comparing the deterioration of renal function and the renal replacement therapy admission, it was noted that...
Overall renal survival at 5 and 10 years, according to histological imaging, is shown in Table 7. Five-year renal survival was 100% in patients with MC, 92.4% in those with DMP and 80.5% for FSGS. At 10 years it was 100%, 89.7% and 44%, respectively, and this difference was statistically significant ($p = 0.001$).

**DISCUSSION**

The treatment schedule for SRNS in children that has been applied in the CMN SXXI HP during the past 15 years seems to be appropriate. Only 10.9% of the patients have presented renal function impairment, onset of renal replacement therapy or death. At 10 years, renal survival is 87.4%.

Treatment for SRNS that has been reported with the most frequency is cyclosporin. There are studies that reported a remission up to 85% of the cases at one year of treatment. In this study, the first line of treatment was with cyclosporin provided that no deterioration of both were more frequent in patients with FSGS (20.4%), compared with those of MC (3%) and DMP (5.5%) (Table 5). This was statistically significant ($p = 0.004$). When analyzing the risk of developing kidney failure according to the initial histological image, it was determined that patients with FSGS presented with a higher risk than with DMP (OR 4.7, 95% CI 1.35–16.39, $p = 0.014$); the same for patients with FSGS when compared with those with MC (OR 8.72, CI 1.045–72.27, $p = 0.036$) (Table 6).

### Table 3. Treatment response of children with SRNS

<table>
<thead>
<tr>
<th>Treatment response</th>
<th>$n$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total remission</td>
<td>108</td>
<td>69.2</td>
</tr>
<tr>
<td>Partial remission</td>
<td>14</td>
<td>9.0</td>
</tr>
<tr>
<td>Active</td>
<td>17</td>
<td>10.9</td>
</tr>
<tr>
<td>Deterioration of renal function</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>Renal substitution therapy</td>
<td>9</td>
<td>5.8</td>
</tr>
<tr>
<td>Death</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 4. Overall renal survival in accordance with treatment in children with SRNS

<table>
<thead>
<tr>
<th>Survival</th>
<th>$n$</th>
<th>Median (months) (95% CI)</th>
<th>5 years</th>
<th>10 years</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>156</td>
<td>150.1 (137.8-162.4)</td>
<td>92.9%</td>
<td>87.4%</td>
<td>15</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>92</td>
<td>149.5 (133.2-165.8)</td>
<td>90.0%*</td>
<td>75%*</td>
<td>9</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>27</td>
<td>114.2 (95.0-133.5)</td>
<td>89.5%*</td>
<td>78.3%*</td>
<td>1</td>
</tr>
<tr>
<td>Cyclosporin-cyclophosphamide</td>
<td>26</td>
<td>144.6 (125.5-163.7)</td>
<td>90.9%*</td>
<td>80.3%*</td>
<td>5</td>
</tr>
</tbody>
</table>

*Mantel-Cox test, $p = 0.811$.

### Table 5. Treatment response and evolution of children with SRNS according to histological image

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>DMP</th>
<th>FSGS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>33</td>
<td>74</td>
<td>44</td>
<td>151</td>
</tr>
<tr>
<td>$n$ (%)</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Remission</td>
<td>27 (79.8)</td>
<td>64 (86.6)</td>
<td>26 (59.1)</td>
<td>117 (77.5)</td>
</tr>
<tr>
<td>Active</td>
<td>5 (15.2)</td>
<td>5 (6.8)</td>
<td>7 (15.9)</td>
<td>17 (11.3)</td>
</tr>
<tr>
<td>Deterioration of renal function</td>
<td>1 (3)</td>
<td>1 (1.4)</td>
<td>3 (6.8)</td>
<td>5 (3.3)</td>
</tr>
<tr>
<td>Admission to substitution therapy</td>
<td>0</td>
<td>3 (4.1)</td>
<td>6 (13.6)</td>
<td>9 (5.9)</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>1 (1.4)</td>
<td>2 (4.5)</td>
<td>3 (2.0)</td>
</tr>
</tbody>
</table>

MC, minimal changes; DMP, diffuse mesangial proliferation; FSGS, focal segmental glomerulosclerosis.
renal function was seen. Our percentage of remission in 92 patients with this medication is 90%, similar to that reported in the literature. The controversial points with this treatment are related with the time of administration and nephrotoxicity. It has been observed that if the administration time is short, there may be relapse. Most of the reported studies report a maximum delivery time of 2 years. In this hospital, the minimum time is 3 years and the treatment is continued up to 1 year of remission without relapses. It is only suspended in case of frequent relapse or if there are data of nephrotoxicity. Of the patients described in this study, the average administration of cyclosporin was 41.7 ± 34.4 months. Therefore, at each consultation, data are reviewed for nephrotoxicity, mainly elevation of nitrogen and, if present, then this treatment is suspended. In this study there was only one case with nephrotoxicity data in 43 control biopsies at 2 years of administration. Renal survival was 90% at 5 years and 75% at 10 years in the group treated with cyclosporin. Although there has been a significant decrease in 10-year renal actuarial survival, this is a projection that depends on the number of patients that are found in this stage of follow-up monitoring. This group has a lower rate of monitoring because most patients are discharged by remission and before 10 years of follow-up care. Therefore, there are data from only 10% of the initial population.

Cyclophosphamide is another medication that has been used in this patient group. The International Study for Kidney Diseases in Children (ISKDC)\(^8\) did not report a better response in patients with steroid-resistant cyclophosphamide. However, in this study, cyclophosphamide was administered orally, whereas in our center it was administered monthly via i.v. bolus. In this study the response with cyclophosphamide is very similar to the group treated with cyclosporin, although the initial state between these groups is not the same. The group administered cyclophosphamide has a poorer prognosis because patients initially presented deterioration of renal function, although the final response is similar, which we consider favorable for the patients.

The group that did not initially respond to cyclosporin but later responded to cyclophosphamide had, during the long term, a similar response to those who responded to only cyclosporin. Apparently, if the patients respond, even if it is with the second scheme, the long-term prognosis is similar to when they initially respond with cyclosporin.

In general terms, the prognosis of renal function in patients with steroid-resistant NS is poor. It has been reported that 30 to 50% of the patients develop renal insufficiency in 10 years.\(^8\) In 1990, the French Group of Pediatric Nephrologists, with their treatment regimens, reported renal survival in 62 cases of 65% at 5 years and of 50% at 10 years.\(^1\) In contrast, we report here that renal survival in 156 patients with the mentioned treatment schedule was 92.9% at 5 years and 87.4% at 10 years, indicating a much more favorable prognosis.

On the other hand, even when there is a response to steroids, it is considered to be a more important predictor than histological image. In this study we found that patients with a histopathological diagnosis of FSGS in the initial biopsy had 4.7 times greater risk of developing renal failure than with DMP; with a diagnosis of MC the risk is 8.72 times higher. Renal survival in the group of patients with FSGS at 10 years was 44%, similar to

### Table 6. Risk of loss of renal function and development of renal insufficiency in children with SRNS in accordance with histological image

<table>
<thead>
<tr>
<th>Histological image</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC vs. DMP</td>
<td>0.18</td>
<td>0.199-17.2</td>
<td>1.000</td>
</tr>
<tr>
<td>FSGS vs. DMP</td>
<td>4.70</td>
<td>1.35-16.39</td>
<td>0.014</td>
</tr>
<tr>
<td>FSGS vs. MC</td>
<td>8.72</td>
<td>1.045-72.27</td>
<td>0.036</td>
</tr>
</tbody>
</table>

### Table 7. Renal survival in accordance with initial histological image in children with SRNS

<table>
<thead>
<tr>
<th>Histological image</th>
<th>n</th>
<th>Median (months) (95% CI)</th>
<th>5 years</th>
<th>10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>33</td>
<td>139.5 (136.0-143.0)</td>
<td>100%</td>
<td>9%</td>
</tr>
<tr>
<td>DMP</td>
<td>74</td>
<td>164.5 (153.2-175.8)</td>
<td>92.4*</td>
<td>89.7*</td>
</tr>
<tr>
<td>FSGS</td>
<td>44</td>
<td>116.5 (89.8-143.4)</td>
<td>80.5%*</td>
<td>44%*</td>
</tr>
</tbody>
</table>

*Mantel and Cox test, \(p = 0.001\).

SRNS, steroid-resistant nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; MC, minimal changes; DMP, diffuse mesangial proliferation.
the 50% reported by Ingulli and Tejani.9 Therefore, it is considered that the group of patients with SRNS with initial histological image of FSGS have a poor prognosis, both for presenting a lower treatment response as well as having a higher percentage of patients who will progress to CRI.

In conclusion, initial treatment of SRNS with cyclosporin in children is adequate and similar to that reported in the literature. The use of cyclophosphamide in patients with impaired renal function or with lack of response to cyclosporin achieved a remission and renal survival similar to that seen with cyclosporin. Histological image of renal biopsy remains to be a prognostic factor for the development of renal failure in this group of patients.

REFERENCES

Guidelines to authors

Boletín Médico del Hospital Infantil de México is the official publication of the Hospital Infantil de México “Federico Gómez.” The journal has been continuously published on a bi-monthly basis since 1944 and publishes studies relating to pediatrics according to the following areas: biomedical, clinical, public health, clinical epidemiology, health education and clinical ethics. The following guidelines are in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals,” published by the International Committee of Medical Journal Editors (http://www.icmje.org).

Types of articles
Types of articles published are as follows: Review Articles, Clinical Case Reports, Clinicopathological Cases, Pediatric Themes and Letters to the Editor. Published articles appear both in print and on-line in Spanish and on-line in English.

Submissions should be sent via electronic mail to: bolmedhim@yahoo.com.mx with the following requirements:
1. Letter addressed to Dr. Gonzalo Gutiérrez Trujillo, Editor, Boletín Médico del Hospital Infantil de México, Departamento de Ediciones Médicas. The letter, signed by the corresponding author, should include the following information:
   a) The enclosed article is being submitted for evaluation for eventual publication in Boletín Médico del Hospital Infantil de México.
   b) The authors declare that the work has not been previously published, has not been previously accepted for publication and has not been submitted simultaneously to another publication.
   c) Type of study should be indicated along with the corresponding pertinent area of pediatrics.
   d) Confirm that the Guidelines to Authors have been reviewed and adhered to prior to submission.
   e) If applicable, the authors should declare any conflict of interest or submit a statement that no conflict of interest exists. In the event of a conflict of interest, the authors must disclose any external financial or economic interest.
   f) All external funding sources should be clearly indicated.
   g) All authors must affirm that they are in agreement with the submission of the manuscript and that they have reviewed and approved the work.

Please be reminded that without the appropriately signed author letter, the initial editorial process will be delayed.

Manuscript preparation
All manuscripts should be prepared with standard programs (Word 97 or higher) using the word processor function of your computer. Double space all sections of the manuscript including References, Tables and Figure Legends. Do not justify right margins and use one-inch margins all around. Keep formatting to a minimum. Pages should be numbered consecutively beginning with the first page and numbers should appear in the lower right corner of the page.

Abbreviations
Complex terms used frequently in the manuscript may be abbreviated. Abbreviations are placed in parentheses at first use in the abstract and again at first use in the text. Confirm that any abbreviations used in Tables are appropriately spelled-out in the Table legend underneath.

Organization of the manuscript
The first page should include the following:
   a) Title of the manuscript (Spanish and English)
   b) Type of manuscript: Original Article, Review Article, Clinical Case Report, etc.
   c) Name(s) of all authors in their order of appearance in relation to the publication
   d) Affiliation of each author (degrees and honors should be omitted)
   e) Name, E-mail address, postal address and telephone number of the corresponding author to whom any correspondence can be directed during the review process of the manuscript.

The second page should contain the Abstract. Note that the Abstract is the most-often read part of the manuscript. For this reason, it must be clear, concise and contain information relevant to the article. Do not use references in the Abstract. In the case of Original Articles and Clinical Case Reports, the Abstract should be structured according to the following sections: Background, Methods, Results, Conclusions, or Background, Clinical Case and Conclusions. Abstracts for Review Articles and Pediatric Themes should be unstructured and include only one paragraph. The Abstract should be limited to 200 words and include only relevant aspects of each of the principal sections of the body of the manuscript. Authors should provide 3–6 keywords following the Abstract and use Medical Subject Headings (MeSH) terms as a guide. Visit: http://www.nlm.nih.gov/mesh/meshhome.html.

The manuscript should include the following sections:
1) Original articles: Introduction, Methods, Results, Discussion and References.
2) Clinical case reports: Introduction, Clinical case, Discussion and References.
3) Clinicopathological cases: Clinical case, Discussion and References.

An Acknowledgment section may be included directly following the Reference section. Granting institutions or other financial aid may be listed under Acknowledgments. If there are persons, other than the authors, who assisted with the study or preparation of the manuscript (i.e., technicians, nurses, ancillary health personnel, editorial assistance), they may be listed here.

References
References should be numbered consecutively, double spaced, and listed in the order in which they appear in the text using Arabic numbers (in the text, references are indicated by superscript Arabic numbers after any punctuation). The Reference section should follow the last section of the manuscript. It is not necessary to begin the References on a separate page. The references should be formatted according to the instructions from the U.S. National Library of Medicine. Abbreviated journal names should reflect the style of Index Medicus (visit http://www.nlm.nih.gov/tsd/serials/lji.html) When a reference cites six or fewer authors, names should be included for all authors. When there are seven or more authors, use the first six names followed by et al. Authors are responsible for the accuracy of references. Please use the following examples for presentation of references:
Journals:
- Published book:
- Book chapter:
- Internet consult:

Tables and Figures All tables and figures including schemes, diagrams and table legends must be presented in an editable form. Do not “copy and paste” material from external sources.

Tables
Tables should be numbered using arabic numbers in the order in which they are cited in the text and include a short descriptive title. Tables should not reiterate information presented in the Results section. For preparation of Tables containing only data, use the “Table Editor” function of your word processing program. Do not insert any vertical lines. Use horizontal lines only for clarity of information under table headings. Confirm that information provided below each table heading is properly aligned and clearly identifiable. Tables containing schemes or diagrams should be prepared in PowerPoint; graphics with shading, bars, dispersions, etc. should be prepared using Excel. Each Table should be prepared on a separate page, following the Reference section. Table footnotes should include any abbreviations that need to be explained and notes relating to the Table should be presented alphabetically using superscript letters.

Figures
Authors should number figures in the order in which they appear in the text. Figures include graphs, charts, photographs, and illustrations. Each figure should be accompanied by a self-explanatory legend. Digital images should be legible and printed with a resolution of not less than 300 dpi, using .jpg (jpeg) or .bmp extension.

Permission to use previously published Tables or Figures
If the manuscript contains Tables or Figures that have been previously published, permission must be obtained from the original Publisher in order to reproduce the material. This applies to Tables or Figures that have been modified, adapted or translated. A sample “permission” letter is available from the Editorial Office of Boletín Médico del Hospital Infantil de México. Appropriate credit must be written per the following example:


Ethical approval of studies and informed consent
In regard to possible ethical conflicts, the rights of patients of privacy and confidentiality shall be maintained. For studies involving human subjects, state the manner in which informed consent was obtained from the study participants (i.e., oral or written). All manuscripts reporting data from studies involving humans or animals are subject to formal review and approval by an appropriate institutional review board or ethics committee and should be described at the end of the Methods section. For investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki as well as in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council) should be followed.

Review process
The first review of the manuscript is performed by the Editor to assure that the manuscript corresponds to the theme of the journal and that all required information has been properly submitted in accordance with the Guidelines to Authors. The second review is carried out by two independent reviewers who have been assigned to the manuscript on the basis of their field of expertise. The identities of authors’ and reviewers’ are kept confidential.

Copyright
All accepted manuscripts become the permanent property of the Boletín Médico del Hospital Infantil de México and may not be published elsewhere, in whole or in part, without prior written permission from the Editor and appropriate credit to the authors and to Boletín Médico del Hospital Infantil de México. Upon acceptance of an article for publication in Boletín Médico del Hospital Infantil de México, a letter signed by all authors shall be submitted transferring copyright of the published article to Boletín Médico del Hospital Infantil de México. If the author(s) wishes to reprint any of the material already published in Boletín Médico del Hospital Infantil de México, prior authorization is required from the Editor.

Note: For periodic up-to-dates of "Guidelines to Authors", please consult our internet page: www.himfg.edu.mx