GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(REVIEW ARTICLE)

Check for updates

Infertility associated with chemotherapy and other physical factors

Alejandra Daniela Garcia Rios [1], Mitzy Alejandra Gonzalez Rivera [1], Bryan Uriel Murguia Valencia [1], María Fernanda Salazar Manzanera [1,*], Natalia Andrea Valdez Vargas [1] and Brissia Lazalde Medina [2]

[1] *Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Dgo.*
[2] *Departamento de genética, Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Dgo.*

GSC Advanced Research and Reviews, 2025, 23(02), 013-019

Publication history: Received on 19 March 2025; revised on 01 May 2025; accepted on 03 May 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.23.2.0122

Abstract

Advances in cancer treatment have improved survival rates and quality of life for survivors, but often come with the side effect of reduced sperm count or azoospermia, which can be temporary or permanent. Radiation and certain drugs are likely to cause a reduction in sperm count in the long term. However, newer drugs derived from natural sources have only modest benefits, particularly on the endocrine aspects of the male reproductive system. This article aims to assess the effects of chemotherapy on infertility and explore measures to minimise its impact. In addition to cytotoxic drugs, other drugs may affect male fertility through various mechanisms, such as altering hormone levels or inducing sexual dysfunction and alterations in spermatogenesis.

Keywords: Cancer; Chemotherapy; Spermatogenesis; Azoospermia; Infertility

1. Introduction

Spermatogenesis is a complex and delicate process that starts in the spermatogonial stem cells (SSCs) located at the base of the seminiferous tubules and ends up producing mature virile gametes. One part of the SSCs is in an inactive state, while another part of these cells is in constant proliferation; this mechanism keeps the number of stem cells stable. These cells go through a series of isolation processes, from spermatogonia to primary spermatocytes and also to secondary spermatocytes, which develop into spermatids, which eventually transform into spermatozoa (1). Testicular cells, especially SSCs and spermatogonia in the proliferative phase and isolation and spermatocytes in meiosis during the process of spermatogenesis, are sensitive to chemotherapy. In most cases, after chemotherapy, sperm quality decreases drastically even for months (2).

It is important to note that chemotherapy can affect testicular development, causing alterations in spermatogenesis and can damage the testes at any stage of life, including development. However, in many cases, it remains difficult to predict the likelihood of an individual developing reproductive problems (3, 4).

Although radiotherapy and chemotherapy are generally effective treatments against malignant neoplasms, they cause damage to the gonads. However, spermatogenesis can continue to occur several times if the spermatogonial cell population is not completely depleted. On the other side, if a stem cell population survives cancer treatment, spermatogonia may continue to regenerate for some time (5).

From an embryological standpoint, chemotherapy and radiotherapy used in the treatment of cancer can cause temporary, permanent or indefinite gonadal damage (6).

* Corresponding author: Salazar Manzanera María Fernanda.

These cytotoxic treatments have a significant impact on a case's ability to have its own natural sperm, which is of particular concern in cases of cancer during fertile periods. In many cases, the ability to achieve pregnancy may be temporarily reduced, forcing a delay in reproduction. Although, permanent sterility may occur (7).

However, it is not only chemotherapy drugs that may be involved, but there are several conditions that interfere with spermatogenesis and reduce sperm production and quality that can cause azoospermia, such as an underlying disease or an obstruction of the reproductive tract (8).

1.1. Chemotherapy

Alkylating agents, the most widely used cancer chemotherapeutic drugs, damage DNA and interfere with cell replication, exerting their cytotoxic effects by forming covalent bonds between their alkyl groups and various nucleophilic molecules present in cells, in particular the nitrogenous bases of DNA. In this way, they prevent cellular DNA replication and RNA transcription, thus inhibiting mitosis and protein synthesis. They act throughout the cell cycle, but are most active in rapidly dividing cells. Many drugs, especially alkylating agents, cause damage to the reproductive organs. Certain cancer chemotherapies or bone marrow transplant drugs have been shown to cause male infertility by azoospermia **(Table 1)** (9,10).

Table 1 Antineoplastic Agents that can Cause or Add to Prolonged Azoospermia in Humans (10).

Effects	**Agents**	**Mechanism of Action**
Prolonged azoospermia	Chlorambucil	DNA breaks
	Cyclophosphamide	Alkylating
	Procarbazine	Alkylating
	Melphalan	Alkylating
	Cisplatin	DNA cross-link
Agents that cause only temporary reductions in sperm count	Adriamycin	DNA intercalating
	Thiotepa	Alkylating
	Cytosine arabinoside	Nucleoside analog
	Vinblastine	Microtubule inhibitor

One such example is busulfan, a chemotherapy drug used to treat certain types of cancer such as chronic myeloid leukemia and polycythemia vera. Unlike other chemicals that primarily target mature sperm, busulfan is especially effective in eradicating spermatogonial stem cells without affecting DNA synthesis. Studies have shown that busulfan treatment disrupts spermatogenesis by damaging both germ cells and Sertoli cells (11). Research has revealed that it can trigger apoptosis (cell death) in germ cells by disrupting the c-kit signaling pathway independently of Fas/FasL and p53. Besides, p53 has been identified as a key protein involved in stromal apoptosis, operating through activation of the extracellular signal-regulated kinase/p38 pathway in response to reactive oxygen species (ROS) (12).

Also, busulfan has been identified to damage spermatogenic cells, leading to up-regulation of tumor necrosis factor (TNFα) and macrophage chemotactic protein (MCP1) expression in Sertoli cells. This upregulation facilitates macrophage infiltration into the testis (13).

Other chemotherapeutic agents that cause secondary male infertility are mechlorethamine or procarbazine in high doses (14). In addition to chemotherapy drugs, there are other drugs that can cause male infertility through various mechanisms **(Table 2)** (15).

Table 2 Other drugs that may cause male infertility (13)

Agents	Effects	Mechanism of action
Immunosuppressants	Decreases sperm count, motility and sperm morphology Reduces the number of spermatocytes, spermatids and affects the expression of the StAR protein (involved in testosterone synthesis).	Reversible seminiferous tubule dystrophy and blockage of spermatogenesis in spermatogonia.
Anti-inflamatory drugs and salicylates	Alterations of sperm parameters (sperm count, motility and morphology).	Active metabolites cause toxic effects through oxidative stress on immature spermatozoa, decreasing their quality.
Opiates	Prolactin secretion by inhibition of dopaminergic cells and reduction of testosterone.	Hypogonadotropic hypogonadism, decreased testosterone and central suppression of GnRH secretion by inhibition of the hypothalamic-pituitary axis.
α-Bloquers	Alterations in semen quality and ejaculatory disorders such as retrograde ejaculation.	It is thought to be related to the affinity of a-blockers for serotonin and dopamine receptors at the central level.
Diuretics	Anti-androgenic effects by inhibiting testosterone synthesis, causing erectile dysfunction and decreased libido.	Inhibition of adrenal and testicular cytochrome P450 and blockade of androgen binding to target cells by receptor competition.

2. Types of chemotherapies

The objective of chemotherapy is to destroy neoplastic cells while protecting and preserving normal cells. However, the majority of chemotherapeutic agents act in a nonspecific manner, causing damage to both malignant and normal cells (16,17).

Classifying chemotherapy into alkylating and non-alkylating chemotherapy, within alkylating therapy, with the participation of drugs such as procarbazine and cyclophosphamide, there are usually harmful effects that severely affect the gonads causing an absence of spermatozoa in the semen (18,19,20).

The oldest and most widely used antineoplastic agents are the so-called alkylating agents, which interact chemically with DNA in addition to their activity in the cell division process. In chemotherapy, multiple targets are defined that can be analyzed in order to create new anti-tumour drugs. Since DNA is one of the most studied targets, it is an ideal subject for further research (21-24).

In non-alkylating types of chemotherapy such as doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD), it causes less harmful effects as few patients would present a transient azoospermia. In groups of people aged 15-35 years, non-alkylating chemotherapy has a very low level of toxicity unlike alkylating chemotherapy (25,26).

2.1. Risk of infertility after chemotherapy

Currently, children and adolescents diagnosed with cancer are undergoing new fertility preservation strategies prior to initiating cancer treatment. The various factors that may contribute to a risk of infertility will depend on the type of treatment administered, as well as the malignancy and the age of the individual (27).

Extensive research has shown that biological children of naturally conceived survivors do not have a high incidence of congenital malformations (28,29), or genetic or chromosomal abnormalities (30,31).

Male patients exposed to alkylating agents and to higher doses of testicular radiation presented a higher risk of fertility alterations, thus explaining that, at high doses of chemotherapy and radiation, there is a greater probability of infertility;

however, there is no dose low enough to guarantee intact fertility, nor a dose high enough to ensure that it produces infertility (27).

2.2. Radiation

Radiation is an important factor that has a great effect on germ cells, damaging them and causing genotoxicity that can be transmitted from generation to generation (32).

The determination of the degree of impact of radiation on spermatogenesis is linked to the intensity and duration of electromagnetic exposure. This not only impairs the male reproductive organs, but also affects the number of sperm produced, their motility, and their ability to bind to oocytes (33).

Among the types of radiation such as electromagnetic radiation that affect conception, is gamma radiation, which also contributes to a negative impact on reproduction (34).

2.3. Fertility preservation in men with cancer.

An effective method for preserving fertility is sperm cryopreservation. Currently, great advances have been made in the storage of prepubertal germ cells by means of testicular tissue and stem cell transplants, which offer great hope for the male gender. Another method is the procurement of male gametes from stem cells, although these are not yet available in humans (35). The descent to low temperatures causes the biological metabolism of cells to slow down significantly, halting enzymatic and chemical reactions (36). Cells and tissues lose osmotic equilibrium with their environment. The extracellular fluid begins to freeze at temperatures of -5°C, but the cytoplasm remains intact (37).

Cryopreservation is recommended prior to treatment initiation for greater efficacy in the event of pregnancy. According to studies, 8 out of 12 patients achieved a successful pregnancy (38). In patients with ejaculatory difficulties or severe oligospermia, an alternative is to perform a testicular biopsy and select the mature or immature testicle protocol based on the presence or absence of spermatozoa. This procedure consists of looking for spermatozoa in the testicles. The method is the same as that used for testicular sperm extraction (TESE) in patients with azoospermia and is therefore called onco-TESTE (39). In addition, prepubertal males who do not yet produce sperm have the option of cryopreserving testicular tissue (TT) or testicular cell suspensions (TST) of spermatogonial stem cells (SSCs) capable of producing sperm from puberty onward to preserve their chances of future reproductive options (40).

Patients with fertility problems such as azoospermia produce a very reduced amount of sperm, which reduces the effectiveness of a successful pregnancy, therefore cryopreservation is a recommended method to protect sperm from any type of toxic aggression that affects their effectiveness, such as radiation and chemotherapy (41).

2.4. Preventive alternatives

Electromagnetic radiation severely affects male fertility, hence antioxidants, anti-free radicals, vitamins such as E and C and melatonin protect sperm cells and thus reduce their damage. In addition to the aforementioned factors, fatty acids, cryptoxanthin and lycopene also play a role in protecting against infertility and supporting more successful reproduction. On the contrary, the consumption of trans fatty acids or low levels of good fatty acids deteriorate its quality (42).

2.5. Other preventive factors

The best way to preserve it would be the implementation of foods containing protein such as fish, low-fat dairy products, fruits and vegetables as a method of prevention for infertility, affecting the same with red meat, caffeine and alcohol (43).

2.6. Spermatogonial stem cells

Spermatogonial stem cells, being the basis of spermatogenesis, by maintaining the process of self-renewal and transmitting genetic information, may be an alternative solution for the regeneration of impaired spermatogenesis.

Their transplantation involves their isolation, cryopreservation of testicular biopsies prior to chemotherapy, followed by intratesticular transplantation of SSCs. However, it is still under investigation (44).

2.7. Genetic effect on the offspring of cancer survivors exposed to chemotherapy

Cancer treatments can impair future reproductive capacity, and various cytotoxic drugs can produce secondary malignant neoplasms due to their mutagenic properties in DNA (45, 46, 47). There is a lack of accurate data regarding treatment exposure due to the rarity of individual genetic disorders. Consequently, the risk of congenital anomalies in offspring related to quantified doses of gonadal radiation is not yet fully understood (48,49).

The Childhood Cancer Survivor Study (CCSS) analyzed pregnancy outcomes in female and male childhood cancer survivors and compared them with a cohort of sibling cancer survivors (50, 51). The study, which compared surviving siblings, revealed that in 1903 pregnancies, 71% resulted in successful births, 1% in fetal deaths, 15% in spontaneous abortions, 12% in miscarriages, and 2% were unknown (48).

A review of the medical literature indicates that cancer survivors and their offspring do not show a considerably increased risk of genetic defects or congenital malformations in the context of chemotherapy or radiotherapy (52-54).

3. Conclusion

Chemotherapy and radiation are treatments used in cancer patients involving drugs that can have side effects on the gonads, which can have a major impact on testicular development or lead to infertility due to azoospermia. In addition, male patients exposed to high doses of radiation had a higher risk of impaired fertility due to germ cell loss. Furthermore, drugs such as busulfan have been shown to damage spermatogenic cells with the involvement of the p53 protein. Finally, cryopreservation is considered to be one of the most effective methods of preserving infertility, as it protects sperm from the side effects caused by physical agents such as chemotherapy and radiotherapy; it is also important to note the preservation method that involves searching for sperm in the testicles, using a method similar to testicular sperm extraction (TESE) in patients with azoospermia, better known as onco-TESTE.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Neto FTL, et al. Spermatogenesis in humans and its affecting factors.Seminars In Cell & Developmental Biology. 2016;59:10–26.

[2] O'Flaherty C, et al. Impact of chemotherapeutics and advanced testicular cancer or Hodgkin lymphoma on sperm deoxyribonucleic acid integrity. Fertility and Sterility. 2010;94(4):1374–1379.

[3] Lopes F, et al. Chemotherapy induced damage to spermatogonial stem cells in prepubertal mouse in vitro impairs long-term spermatogenesis.Toxicol Rep. 2020;8:114-123.

[4] Delessard M, et al. Exposure to Chemotherapy During Childhood or Adulthood and Consequences on Spermatogenesis and Male Fertility. Int J Mol Sci. 2020;21(4):1454.

[5] Okada K, Fujisawa M. Recovery of Spermatogenesis Following Cancer Treatment with Cytotoxic Chemotherapy and Radiotherapy.World J Mens Health. 2019;37(2):166-174.

[6] Rivkees S.A., Crawford J.D. The relationship of gonadal activity and chemotherapy-induced gonadal damage. JAMA. 1988;259:2123–2125.

[7] Hermann BP, et al. Spermatogonial stem cells in higher primates: are there differences from those in rodents? Reproduction. 2010;139:479–93.

[8] Cocuzza M, Alvarenga C, Pagani R. The epidemiology and etiology of azoospermia.Clinics (Sao Paulo). 2013;68 Suppl 1:15–26.

[9] Benedí J, Gómez del Río MÁ. Antineoplastic drugs (I). Farm Prof 2006; 20(2):60-5.

[10] Meistrich ML, et al. Cancer: Principles and Practice of Oncology. 7th ed. Lippincott Williams & Wilkins; Philadelphia: 2005. pp. 2560–74

[11] Buggia I., et al. Ann. Pharmacother. 1994;28:1055–1062.

[12] Choi Y.J., et al. Murine male germ cell apoptosis induced by busulfan treatment correlates with loss of c-kit-expression in a Fas/FasL. and p53-independent manner. FEBS Lett. 2004;575:41–51.

[13] Zhang X., et al. spermatogenic cells induce inflammatory gene expression in mouse Sertoli cells through the activation of Toll-like receptors 2 and 4. Mol. Cell. Endocrinol. 2013;365:162–173.

[14] 1.Heredia Benito M, et al. Drugs and infertility: review. Clinical and Research in Gynecology and Obstetrics. 2012; 39(2):69–72.

[15] Semet M, Paci M, Saïas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H, et al. The impact of drugs on male fertility: a review. Andrology. 2017;5(4):640–63.

[16] Murad, AM; Katz, A.; Bases Clínicas del Tratamiento Oncológico ; Guanabara; Río de Janeiro, pág. 41.

[17] Salmonm, SE en farmacología clínica y básica , Katzung, BG, ed.; Guanabara Koogan SA: Río de Janeiro, 1998, p. 629-655.

[18] Karati D, et al. Alkylating Agents, the Road Less Traversed, Changing Anticancer Therapy. Anticancer Agents Med Chem. 2022;22(8):1478-1495.

[19] Singh RK, Kumar S, Prasad DN, Bhardwaj TR. Therapeutic journery of nitrogen mustard as alkylating anticancer agents: Historic to future perspectives. Eur J Med Chem. 2018 May 10;151:401-433.

[20] More GS, et al. Nitrogen Mustards as Alkylating Agents: A Review on Chemistry, Mechanism of Action and Current USFDA Status of Drugs. Anticancer Agents Med Chem. 2019;19(9):1080-1102.

[21] Keskin, O, et al.; Anti-Cancer Drug Des. 2000, 15, 79.

[22] Iida H, Jia G, J William Lown. Rational recognition of nucleic acid sequences. Current opinion in biotechnology. 1999 Feb 1;10(1):29–33.

[23] Dervan P. Molecular recognition of DNA by small molecules. Bioorganic & Medicinal Chemistry. 2001 Sep;9(9):2215–35.

[24] Krugh TR. Drug-DNA interactions. Current Opinion In Structural Biology. 1994;4(3):351-64.

[25] van der Kaaij MA, et al. Fertility preservation after chemotherapy for Hodgkin lymphoma. Hematol Oncol. 2010 Dec;28(4):168-79.

[26] Zraik IM, Heß-Busch Y. Management von Nebenwirkungen der Chemotherapie und deren Langzeitfolgen [Management of chemotherapy side effects and their long-term sequelae]. Urologe A. 2021 Jul;60(7):862-871.

[27] Klipstein S, et al. Fertility Preservation for Pediatric and Adolescent Patients With Cancer: Medical and Ethical Considerations.Pediatrics 2020;145(3).

[28] Seppänen VI, Artama MS, Malila NK, Pitkäniemi JM, Rantanen ME, Ritvanen AK, et al. Risk for congenital anomalies in offspring of childhood, adolescent and young adult cancer survivors. International Journal Of Cancer. 2016;139(8):1721-30.

[29] Green DM, et al. Congenital Anomalies in Children of Patients Who Received Chemotherapy for Cancer in Childhood and Adolescence.New England Journal Of Medicine/The New England Journal Of Medicine. 1991;325(3):141-6.

[30] Winther JF, et al. Chromosomal Abnormalities among Offspring of Childhood-Cancer Survivors in Denmark: A Population-Based Study.American Journal Of Human Genetics. 2004;74(6):1282-5.

[31] Byrne J, et al. Genetic Disease in Offspring of Long-Term Survivors of Childhood and Adolescent Cancer. American Journal Of Human Genetics. 1998;62(1):45-52.

[32] Fukunaga H, et al. A Brief Overview of Radiation-Induced Effects on Spermatogenesis and Oncofertility. Cancers (Basel). 2022;14(3):805.

[33] Zha XD, et al. Impacts of electromagnetic radiation from cellphones and Wi-Fi on spermatogenesis. Zhonghua Nan Ke Xue. 2019;25(5):451-455.

[34] Hasaballah AI, et al. Impact of paternal transmission of gamma radiation on reproduction, oogenesis, and spermatogenesis of the housefly, Musca domesticaL. (Diptera: Muscidae). Int J Radiat Biol. 2021;97(3):376-385.

[35] Tournaye H, Dohle GR, Barratt CLR. Fertility preservation in men with cancer. Lancet. 2014;384(9950):1295–301.

[36] Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Human Reproduction Update. 2016;22(6):744-61.

[37] Mazur P. Cryobiology: The Freezing of Biological Systems.Science. 1970;168(3934):939-49.

[38] Takeshima T, Mimura N, Aoki S, Saito T, Karibe J, Usui K, et al. Pre- and post-chemotherapy spermatogenesis in male patients with malignant bone and soft tissue tumors. Frontiers In Pharmacology. 6 de diciembre de 2023;14.

[39] Picton HM, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys.Human Reproduction. 2015;30(11):2463-75.

[40] Goossens E, Van Saen D, Tournaye H. Spermatogonial stem cell preservation and transplantation: from research to clinic.Human Reproduction. 2013;28(4):897-907.

[41] Liu S, Li F. Cryopreservation of single-sperm: where are we today? Reprod Biol Endocrinol. 2020;18(1):41.

[42] Gaskins AJ, Chavarro JE. Diet and fertility: a review. Am J Obstet Gynecol. 2018 Apr;218(4):379-389.

[43] Salas-Huetos A, Bulló M, Salas-Salvadó J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. Hum Reprod Update. 2017 Jul 1;23(4):371-389

[44] Abdelaal NE, Tanga BM, Abdelgawad M, Allam S, Fathi M, Saadeldin IM, Bang S, Cho J. Cellular Therapy via Spermatogonial Stem Cells for Treating Impaired Spermatogenesis, Non-Obstructive Azoospermia. Cells. 2021 Jul 14;10(7):1779. doi: 10.3390/cells10071779. PMID: 34359947; PMCID: PMC8304133.

[45] Fossa SD. Parenthood in Survivors After Adulthood Cancer and Perinatal Health in Their Offspring: A Preliminary Report. Journal Of The National Cancer Institute Monographs 1 de marzo de 2005;2005(34):77-82

[46] Sankila R, Olsen JH, Anderson H, Garwicz S, Glattre E, Hertz H, et al. Risk of Cancer among Offspring of Childhood-Cancer Survivors. New England Journal Of Medicine/The New England Journal Of Medicine. 7 de mayo de 1998;338(19):1339-44.

[47] Signorello LB, Mulvihill JJ, Green DM, Munro HM, Stovall M, Weathers RE, et al. Congenital Anomalies in the Children of Cancer Survivors: A Report From the Childhood Cancer Survivor Study. Journal Of Clinical Oncology. 20 de enero de 2012;30(3):239-45.

[48] Nagarajan R. Pregnancy Outcomes in Survivors of Childhood Cancer. Journal Of The National Cancer Institute Monographs. 1 de marzo de 2005;2005(34):72-6.

[49] Hudson MM. Reproductive Outcomes for Survivors of Childhood Cancer. Obstetrics And Gynecology (New York 1953 Online)/Obstetrics And Gynecology. 1 de noviembre de 2010;116(5):1171-83.

[50] Green DM, Whitton JA, Stovall M, Mertens AC, Donaldson SS, Ruymann FB, et al. Resultado del embarazo de mujeres sobrevivientes de cáncer infantil: un informe del Estudio de sobrevivientes de cáncer infantil.Am J Obstet Gynecol 2002;187:1070–80.

[51] Green DM, Whitton JA, Stovall M, Mertens AC, Donaldson SS, Ruymann FB, et al. Resultado del embarazo de las parejas de hombres sobrevivientes de cáncer infantil: un informe del Estudio de sobrevivientes de cáncer infantil.J Clin Oncol 2003;21:716–21.

[52] Signorello LB, Mulvihill JJ, Green DM, Munro HM, Stovall M, Weathers RE, et al. Congenital Anomalies in the Children of Cancer Survivors: A Report From the Childhood Cancer Survivor Study. Journal Of Clinical Oncology.20 de enero de 2012;30(3):239-45.

[53] Sankila R, Olsen JH, Anderson H, Garwicz S, Glattre E, Hertz H, et al. Risk of Cancer among Offspring of Childhood-Cancer Survivors. New England Journal Of Medicine/The New England Journal Of Medicine. 7 de mayo de 1998;338(19):1339-44.

[54] Winther JF, Olsen JH, Wu H, Shyr Y, Mulvihill JJ, Stovall M, et al. Genetic Disease in the Children of Danish Survivors of Childhood and Adolescent Cancer. Journal Of Clinical Oncology. 1 de enero de 2012;30(1):27-33.