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IS ROUTINE TORCH SCREENING AND URINE CMV CULTURE WARRANTED IN SMALL FOR GESTATIONAL AGE NEONATES?

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ABSTRACT

Background: Congenital infections are associated with a wide variety of clinical symptoms, including small for gestational age (SGA).

Aims: To determine the co-occurrence of SGA and congenital ToRCH infections, as diagnosed by ToRCH serologic tests and/or cytomegalovirus (CMV) urine culture.

Study design: We performed a retrospective study of all neonates admitted to our neonatal intensive care unit from January 2004 to February 2010 in whom SGA was diagnosed and ToRCH serologic tests and/or CMV urine cultures were performed.

Results: ToRCH serologic tests (in neonatal or maternal serum) and/or a CMV urine culture were performed in 112 neonates with SGA. None of the neonates tested positive for *Toxoplasma gondii*, rubella, and herpes simplex virus. Positive CMV urine culture was detected in 2% (2/112) of neonates, but their CMV IgM titers were negative.

Conclusions: The co-occurrence of ToRCH congenital infection in infants with SGA is rare. Routine ToRCH screening in neonates with isolated SGA does not seem warranted and should be limited to CMV urine cultures.

INTRODUCTION

Neonates whose birth weight is below 2 standard deviations (SD) for their gestational age are termed small for gestational age (SGA) neonates [1-3]. SGA is not a specific disease entity, but a manifestation of many possible disorders and occurs in approximately 3–7% of all neonates [1 4]. Several disorders can lead to SGA, including fetal factors such as genetic conditions and congenital anomalies [1 5-7], placental factors such as pathologies of umbilical cord or placental parenchyma [6 7], and maternal factors related to medical conditions, drug abuse, uterine factors (Figure 3.1) [1 5-10]. Smoking is the single most common cause of impaired fetal growth [7]. Pathology affecting the placenta is responsible for the large majority of SGA [2 5].

Fetal
- Chromosomal and genetic disorders (eg. Autosomal trisomies (13,18 and 21)/Triploidy/Unbalanced chromosomal deletions and translocations) [1 5-7]
- Congenital anomalies (eg. Potter's syndrome/ cardiac anomalies) [7]
- Multiparity [1 5 7]

Placental
- Pathologic changes in umbilical cord/ fetal circulation/ vilous parenchyma [6 7]

Maternal
- Medical conditions associated with maternal hypoxemia and/or deficient uteroplacental perfusion (eg. essential hypertension, pre-eclampsia, insulin-dependent diabetes mellitus with vasculopathy, systemic lupus erythematosus, chronic kidney disease and inherited trombophilia) [5-10]
- Therapeutic drugs (eg. opiates, steroids, warfarin and anticonvulsants) [5]

Figure 3.1: Factors associated with fetal growth.

Congenital ToRCH infections have also been reported to be associated with SGA. Besides SGA, congenital ToRCH infections may also lead to a variety of other clinical signs, such as cutaneous manifestations (purpura, jaundice, blueberry muffins), liver disease and cerebral abnormalities, e.g. cerebral calcification, lenticulostriatal vasculopathy and subependymal cysts [11-14].

ToRCH is an acronym which groups several micro-organisms including *Toxoplasma gondii*, rubella, cytomegalovirus (CMV) and herpes simplex virus (HSV). Although each organism may cause distinct clinical features, ToRCH testing is often requested as 'one' serologic diagnostic test [13 15].

Since congenital infections are one of the possible underlying pathologic processes linked to SGA, some authors have suggested that ToRCH screening should be part of the routine diagnostic work-up in SGA neonates [5 16-18]. However, the co-occurrence of congenital infections and SGA is merely speculative and based on limited data [18].

The objective of our study was to evaluate the co-occurrence of congenital ToRCH infections in a larger series of neonates with SGA.

METHODS

All consecutive newborns admitted to the neonatal intensive care unit (NICU) of the Leiden University Medical Centre between January 2004 and February 2010 were eligible for the study. The Leiden University Medical Centre is a tertiary care centre in The Netherlands. We searched our computerized database for all SGA neonates and recorded whether ToRCH serologic tests and/or a CMV urine culture were performed. A local guideline for diagnostic management at birth in SGA neonates was not available during the study period. Diagnostic investigation for the presence of congenital ToRCH infections was therefore not routinely performed in all SGA neonates but left to the judgement of the attending neonatologist. Both the results of maternal and neonatal ToRCH tests were recorded. If evaluation of an SGA neonate at birth revealed that maternal ToRCH tests had already been performed during pregnancy, repetition of ToRCH serologic tests in neonatal serum was not required. ToRCH serologic tests and/or urine CMV culture had to be performed in the period of 6 months before birth up to 3 weeks after birth with maternal and/or neonatal sera and urine. For this study, SGA was defined as a birth weight below 2 SD according to the growth charts for the Dutch population [19].

Medical charts were reviewed for the presence of clinical findings and cranial ultrasound abnormalities associated with ToRCH infections such as liver disease, cutaneous manifestations and cerebral abnormalities including hydrocephaly, calcifications, subependymal cysts and lenticulostriatal vasculopathy. Cranial ultrasound, used to identify cerebral abnormalities associated with ToRCH infections, was performed with an Aloka 5000 scanner (Biomedic Nederland B.V., Almere, The Netherlands) with a multifrequency transducer (set at 7.5 MHz).

Neonatal and maternal charts were reviewed for the presence of medical conditions associated with SGA such as genetical or syndromal abnormalities of the neonate, twin pregnancy, single umbilical artery, pregnancy induced hypertension, pre-eclampsia, therapeutic drug use, nicotine abuse, illicit drug abuse, maternal age, nulliparity, and several maternal diseases including diabetes mellitus with vasculopathy, systemic lupus erythematosus, chronic kidney disease, chronic hypertension, irritable bowel disease and thrombophilia.

The test types used for ToRCH serology were: IgM *Toxoplasma*, IgM Rubella (both μ-capture) and IgM CMV (indirect EIA) were tested using Vidas enzyme-linked fluorescent assays (bioMérieux, Marcyl'Etoile, France). IgM HSV was tested using the Merifluor IFA

(Meridian Bioscience, Cincinnati, OH). As from July 2007 IgM HSV testing was performed using the indirect EIA from Virion\Serion (Wűrzburg, Germany). CMV urine cultures were performed using MRC-5 shell-vials (LGC Standards, Teddington, Middlesex, UK). After 18–24 h incubation at 37°C, cells were stained with an indirect immunofluorescence assay using a monoclonal antibody against p72 CMV antigen (clone 13, Argene Biosoft, France).

Statistical analysis

Descriptive analyses on maternal and neonatal data were performed. To determine the differences between the group of infants with SGA with and without investigations for congenital infections, we used Fisher's exact test (for categorical variables). A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

During the study period, 3,552 infants were admitted to our NICU. SGA was diagnosed in 171 (5%) infants. ToRCH serologic tests and/or a CMV urine culture were performed in 65% (112/171) of cases. The clinical characteristics of the study population are listed in Table 3.1. Diagnostic investigation for the presence of ToRCH infections in SGA neonates was not routinely performed in all neonates but left to the judgement of the attending neonatologist. We found no statistical significant differences in clinical characteristics between the groups with and without investigations.

Table 3.1: Clinical characteristics of 112 neonates with SGA in whom ToRCH serology and/or CMV urine was performed

Gestational age at birth – weeks[a]	34 ± 4
Male – n (%)	63 (56%)
Birth weight – gram[a]	1,427 ± 640
Head circumference < 2 SD[28] – n (%)	67/103 (65%)
Hydrocephaly – n (%)	1 (1%)
Subependymal cysts – n (%)	3 (3%)
Lenticulostriatal vasculopathy – n (%)	2 (2%)
Neonatal sepsis – n (%)	27 (24%)

[a] Mean ± standard deviation.

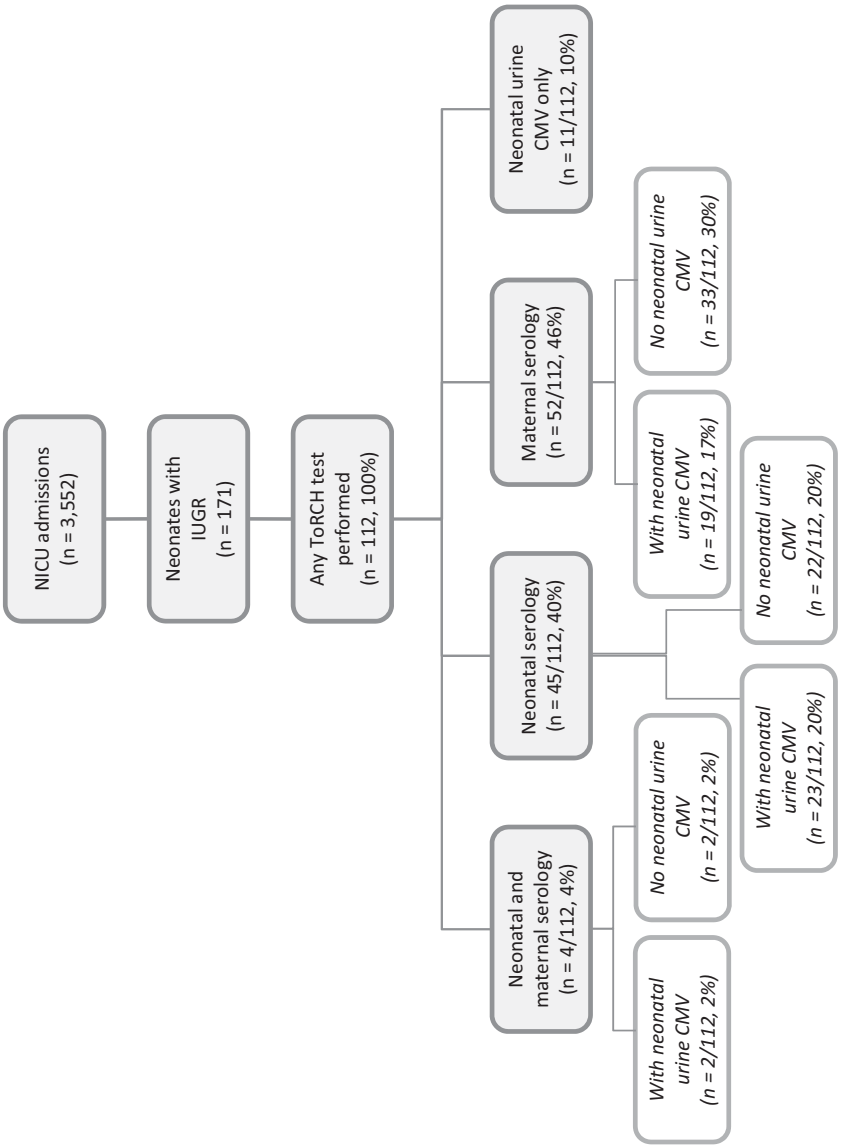

Figure 3.2: Flowchart of the type of ToRCH tests used in the study population.

Different combinations of tests for congenital infections were used. ToRCH serologic tests in either maternal or neonatal serum were performed in 90% (102/112) of the cases. Neonatal serum was used for testing in 40% (45/112) and maternal serum in 47% (53/112). In 4% (4/112) of the cases, both neonatal and maternal serum were used. Urine CMV cultures were performed in 49% (55/112) of the newborns. IgM titers for *Toxoplasma gondii*, Rubella, CMV and HSV were negative for all infants and mothers. Two neonates had a positive CMV urine culture, but a negative CMV IgM titer. Both infants had a symmetrical growth restriction and isolated SGA without other clinical signs of congenital CMV infection. Detailed information on the diagnostic work-up used in the study population is depicted in Figure 3.2.

All SGA neonates included in this study had no additional clinical findings suggestive of congenital infection. No infants were found to have hepatosplenomegaly, cutaneous manifestations or cerebral calcifications. Six neonates had abnormalities on cranial ultrasound scans which have been suggested to be associated with congenital ToRCH infection including subependymal cysts (n = 3), lenticulostriatal vasculopathy (n = 2) and hydrocephaly (n = 1). The infant with hydrocephaly was a donor twin treated with laser surgery due to twin–twin transfusion syndrome (TTTS).

Review of maternal and neonatal charts revealed that in 64% (72/112) a condition known to be associated with SGA was present (Table 3.2).

Table 3.2: Factors associated with SGA (n = 112)[a]

Twin pregnancy – n (%)	23 (21%)
Twin-to-twin transfusion syndrome (donor) – n (%)	2 (2%)
Single umbilical artery – n (%)	7 (6%)
ToRCH infection – n (%)	2 (2%)
Genetic and chromosomal abnormalities[b] – n (%)	15 (13%)
Pregnancy induced hypertension – n (%)	7 (6%)
Pre-eclampsia – n (%)	13 (12%)
Maternal disease[c] – n (%)	6 (5%)
Therapeutic drug use[d] – n (%)	8 (7%)
Illicit drug abuse – n (%)	6 (5%)
Nicotine abuse – n (%)	14 (13%)

[a] Conditions may overlap in some cases.
[b] Trisomy 18, trisomy 21, 4q35 deletion, tetralogy of Fallot, Smith Lemli Opitz syndrome, Perlman syndrome, or dysmorphic features without diagnosis.
[c] Systemic Lupus Erythematosus, chronic kidney disease, chronic hypertension and/ or irritable bowel disease.
[d] Opiates, steroids, warfarin and/or anticonvulsants.

DISCUSSION

In this study we found that the co-occurrence of congenital ToRCH infections in neonates with SGA is extremely low. Only 2 of the 112 (2%) infants with SGA had evidence of congenital CMV infection. No evidence of co-occurrence of SGA and *Toxoplasma gondii*, rubella or HSV was found. Our findings question the validity of routine diagnostic investigation for ToRCH congenital infections in SGA neonates.

Caution is required when interpreting these results due to the limited sensitivity of serologic tests for congenital infections [14 20]. Nevertheless, urine CMV cultures are considered a highly reliable test for the presence of CMV infection [14]. Maternal infection leading to intrauterine infection of the foetus can cause SGA [1 5-7 21]. The pathogens include the ToRCH-group: *Toxoplasma gondii*, rubella, CMV and HSV [6 7 21]. More recently, varicella-zoster and human immunodeficiency virus have been suggested as possible pathogens [7]. All congenital infections are uncommon, apart from CMV [1 6 7 21]. Fetal infection should be considered when multiple organ system anomalies, SGA, placental enlargement or abnormalities of the amniotic fluid volume are demonstrated [21]. Infectious diseases account for 5–10% of the SGA cases [4 7], but SGA is rarely an isolated manifestation of congenital infection with ToRCH agents [4].

Extensive search of the literature yielded only a handful of studies on the incidence of congenital infections in SGA neonates. The published studies show conflicting results on the risk of congenital infections, ranging from 0 to 13% (Table 3.3). By pooling together the reported results in studies on congenital infections in SGA, we found that the average rate of congenital ToRCH infections in neonates with SGA is 0.6% (10/1626). Congenital infection was mostly due to CMV infection (5/10), followed by rubella infection (4/10) and toxoplasmosis (1/10). However, care should be taken when interpreting these results because of diversity of performed tests and often incomplete information.

The results of our study should be interpreted in the epidemiological context of congenital infections in The Netherlands. The incidence of congenital infections varies by country and is in general reported to be low in The Netherlands. The prevalence of congenital CMV in the Netherlands is reported to be 0.09% [22]. The prevalence of congenital HSV and *Toxoplasma gondii* in Northern and Western European countries is 0.003% [23 24] and 0.15%, respectively [25]. The incidence of Congenital Rubella Syndrome (CRS) is expected to be low, due to immunity by vaccination. In areas of lower coverage with the rubella vaccine, outbreaks may occur [26]. The rubella status of the mother is routinely tested in the Netherlands at 12 weeks gestation. This makes testing for rubella redundant in asymptomatic newborns.

Table 3.3: Summary of studies on clinical co-occurrence between congenital ToRCH infection and SGA

Author, year [ref]	Neonates with SGA – n	ToRCH tests positive – n (%)	Comments
Matthews TG[a], 1978 [29]	969	3 (0%)	Rubella: n = 2 Toxoplasmosis: n = 1
Commey JO[b], 1979 [30]	71	1 (1%)	CMV: n = 1
Primhak RA, 1982 [18]	23	3 (13%)	Rubella: n = 2 CMV: n = 1
Weiner CP, 1989 [31]	21	0 (0%)	
Vik T[c], 1996 [32]	366	0 (0%)	
Khan NA, 2000 [4]	75	1 (1%)	CMV: n = 1
This study, 2010	112	2 (2%)	CMV: n = 2
Total	1,637	10 (0.6%)	Rubella: n = 4 Toxoplasmosis: n = 1 CMV: n = 5

[a] Only IgM in umbilical cord blood determined.
[b] Only serologic tests performed, no urine CMV cultures.
[c] No detailed information on type and number of tests for congenital infection.

In this study the yield of a workup for ToRCH infection among infants with SGA is low and does not justify the incurred costs. Already in 1982, Primhak et al. reported that the screening policy in SGA neonates had evolved without formal rational justification and stated that clinical investigation of ToRCH infection should be confined to those babies with other clinical evidence of infection co-occurring with SGA [18]. Recent studies on the indication and value of ToRCH testing for other fetal and neonatal indications suggest that ToRCH testing is often not necessary and can be limited to screening for CMV, using urine cultures [4 11-13].

The results of our study should be interpreted with care due to several methodological limitations. Retrospective studies are known to be susceptible for bias. Not all infants with SGA were investigated for congenital infections. However, we found no difference in clinical characteristics between the group with and the group without investigations, suggesting that a selection bias is unlikely to have occurred. In addition, the heterogeneity of the tests (maternal and/or neonatal serologic tests, and urine CMV culture) used may have weakened the study. Moreover, the sensitivity of serologic tests for congenital infections is reported to be limited [14 20]. Nevertheless, urine CMV cultures are considered a highly reliable test for the presence of CMV infection [14].

Whether the risk of CMV infection (2%) reported in this study is increased compared to the general population is unknown. Ideally, the decision to perform routine ToRCH serologic tests in neonates with SGA should be based on well-designed studies (i.e. large, prospective case-

control studies) showing an increased prevalence of congenital infection in this subgroup of neonates compared to a control group. We calculated that in a case-control study a minimum of 2,250 neonates in each study group is required to demonstrate a statistically significant increase in incidence of congenital CMV infection from 1% (controls) to 2% (cases). Given the rarity of SGA, such a study would be extremely difficult to perform.

Importantly, since all SGA infants included in this study had no other associated clinical symptoms related to congenital ToRCH infection, our results are limited to infants with isolated SGA. Whether the 6 SGA neonates with cerebral lesions (subependymal cysts, lenticulostriatal vasculopathy and hydrocephaly) reported in this study should be regarded as isolated SGA neonates, is controversial.

We have recently shown in several studies that there is a dearth of high-level evidence to substantiate the claim that neonates with subependymal cysts or lenticulostriatal vasculopathy could be at increased risk for congenital ToRCH infection. In addition, the infant with hydrocephaly was a donor twin treated with laser surgery due to twin–twin transfusion syndrome, a disease known to be associated with increased risk for cerebral injury. Hydrocephaly in this case was thus almost certainly related to TTTS [27].

In conclusion, we suggest that in countries with a low incidence of congenital infections, complete routine ToRCH screening in neonates with isolated SGA does not seem warranted and should be limited to CMV urine cultures.

REFERENCES

1. Resnik R. Intrauterine growth restriction. Obstet Gynecol 2002;99(3):490-6.
2. Ananth CV, Vintzileos AM. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum Dev 2009;85(10):653-8. doi: 10.1016/j.earlhumdev.2009.09.004
3. Breeze AC, Lees CC. Prediction and perinatal outcomes of fetal growth restriction. Semin Fetal Neonatal Med 2007;12(5):383-97. doi: 10.1016/j.siny.2007.07.002
4. Khan NA, Kazzi SN. Yield and costs of screening growth-retarded infants for torch infections. Am J Perinatol 2000;17(3):131-5. doi: 10.1055/s-2000-9288 [published Online First: 2000/09/30]
5. Vandenbosche RC, Kirchner JT. Intrauterine growth retardation. Am Fam Physician 1998;58(6):1384-90, 93-4. [published Online First: 1998/11/06]
6. Cox P, Marton T. Pathological assessment of intrauterine growth restriction. Best Pract Res Clin Obstet Gynaecol 2009;23(6):751-64. doi: S1521-6934(09)00082-0 [pii] 10.1016/j.bpobgyn.2009.06.006 [published Online First: 2009/10/27]
7. Bernstein PS, Divon MY. Etiologies of fetal growth restriction. Clin Obstet Gynecol 1997;40(4):723-9. [published Online First: 1998/01/16]
8. Barker DJ. In utero programming of chronic disease. Clin Sci (Lond) 1998;95(2):115-28.
9. Villar J, Carroli G, Wojdyla D, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? Am J Obstet Gynecol 2006;194(4):921-31. doi: 10.1016/j.ajog.2005.10.813
10. Srinivas SK, Edlow AG, Neff PM, et al. Rethinking IUGR in preeclampsia: dependent or independent of maternal hypertension? J Perinatol 2009;29(10):680-4. doi: 10.1038/jp.2009.83
11. van der Weiden S, Steggerda SJ, Te Pas AB, et al. Routine TORCH screening is not warranted in neonates with subependymal cysts. Early Human Development 2010;86(4):203-07. doi: 10.1016/j.earlhumdev.2010.02.009
12. de Jong EP, Lopriore E, Vossen AC, et al. Is routine TORCH screening warranted in neonates with lenticulostriate vasculopathy? Neonatology 2010;97(3):274-8. doi: 10.1159/000255166
13. Abdel-Fattah SA, Bhat A, Illanes S, et al. TORCH test for fetal medicine indications: only CMV is necessary in the United Kingdom. Prenatal Diagnosis 2005;25(11):1028-31. doi: 10.1002/pd.1242
14. Gaytant MA, Steegers EA, Semmekrot BA, et al. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet Gynecol Surv 2002;57(4):245-56.
15. Cullen A, Brown S, Cafferkey M, et al. Current use of the TORCH screen in the diagnosis of congenital infection. J Infect 1998;36(2):185-8. [published Online First: 1998/11/20]
16. Lepercq J, Mahieu-Caputo D. Diagnosis and management of intrauterine growth retardation. Horm Res 1998;49 Suppl 2:14-9.
17. Sheridan C. Intrauterine growth restriction--diagnosis and management. Aust Fam Physician 2005;34(9):717-23.
18. Primhak RA, Simpson RM. Screening small for gestational age babies for congenital infection. Clin Pediatr (Phila) 1982;21(7):417-20. [published Online First: 1982/07/01]
19. Kloosterman GJ. [Intrauterine growth and intrauterine growth curves]. Maandschr Kindergeneeskd 1969;37(7):209-25.
20. Best JM, Sutherland S. Diagnosis and prevention of congenital and perinatal infections. BMJ 1990;301(6757):888-9.
21. Degani S. Sonographic findings in fetal viral infections: a systematic review. Obstet Gynecol Surv 2006;61(5):329-36. doi: 10.1097/01.ogx.0000216518.85796.88
22. Gaytant MA, Galama JMD, Semmekrot BA, et al. The incidence of congenital cytomegalovirus infections in The Netherlands. Journal of Medical Virology 2005;76(1):71-75. doi: 10.1002/jmv.20325

23. Poeran J, Wildschut H, Gaytant M, et al. The incidence of neonatal herpes in The Netherlands. J Clin Virol 2008;42(4):321-5. doi: S1386-6532(08)00063-2 [pii] 10.1016/j.jcv.2008.02.004 [published Online First: 2008/03/25]
24. Gaytant MA, Steegers EA, van Cromvoirt PL, et al. [Incidence of herpes neonatorum in Netherlands]. Ned Tijdschr Geneeskd 2000;144(38):1832-6.
25. Jenum PA, Stray-Pedersen B, Melby KK, et al. Incidence of Toxoplasma gondii infection in 35,940 pregnant women in Norway and pregnancy outcome for infected women. J Clin Microbiol 1998;36(10):2900-6.
26. Hahne S, Macey J, van Binnendijk R, et al. Rubella outbreak in the Netherlands, 2004-2005: high burden of congenital infection and spread to Canada. Pediatr Infect Dis J 2009;28(9):795-800. doi: 10.1097/INF.0b013e3181a3e2d5
27. Lopriore E, van Wezel-Meijler G, Middeldorp JM, et al. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006;194(5):1215-20. doi: 10.1016/j.ajog.2005.12.003
28. Nellhaus G. Head circumference from birth to eighteen years. Practical composite international and interracial graphs. Pediatrics 1968;41(1):106-14.
29. Matthews TG, O'Herlihy C. Significance of raised immunoglobulin M levels in cord blood of small-for-gestational-age infants. Arch Dis Child 1978;53(11):895-8.
30. Commey JO, Fitzhardinge PM. Handicap in the preterm small-for-gestational age infant. J Pediatr 1979;94(5):779-86.
31. Weiner CP, Williamson RA. Evaluation of severe growth retardation using cordocentesis--hematologic and metabolic alterations by etiology. Obstet Gynecol 1989;73(2):225-9.
32. Vik T, Vatten L, Markestad T, et al. Morbidity during the first year of life in small for gestational age infants. Arch Dis Child Fetal Neonatal Ed 1996;75(1):F33-7.