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Research Article

Screening for Infection - An Analysis of the Microcirculation in Term Newborns

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Abstract

Microcirculatory disturbances during infection have been described in adults and newborns. However, screening for infection by assessing the microcirculation in newborns has not been successfully established.

In 110 term newborns, microcirculatory parameters were obtained prospectively and consecutively by Sidestream Dark Field imaging at the upper ear conch in the first 72 hours after birth. Infants treated with antibiotics for early newborn infection (group A) were compared to the remaining control group (group C). The quality of flow was analyzed by the standard AVA analysis and by a newly introduced scoring system (bedside score).

Group A displayed a significantly reduced functional vessel density (mean [95% CI]; A: 14.0 [13.6-14.3] vs. C: 14.5 [14.3-14.8] mm/mm^2, $p<0.05$) with a higher proportion of hyperdynamic flow in the AVA analysis (mean [95% CI]; A: 3.2 [2.9-3.5] vs. C: 3.0 [2.9-3.0], $p<0.001$) and abnormal flow in the bedside score (mean [95% CI]; A: 1.2 [0.8-1.7] vs. C: 0.3 [0.2-0.4], $p<0.0001$). Hyperdynamic flow was associated with a fivefold increased risk for infection.

Conclusion

Neonates with infection have a reduced FVD and hyperdynamic flow early on. The bedside score showed congruent results and could facilitate identification of infants at risk for infection.

Abbreviations

A: Antibiotic Group
AVA: Automated Vascular Analysis
C: Control Group
CRP: C-Reactive Protein
FVD: Functional Vessel Density
LED: Light Emitting Diode
MFI: Microvascular Flow Index
PC: Personal Computer
R^2: Coefficient of Determination
TVD: Total Vessel Density
SDF: Sidestream Dark Field
VS: Vessel Surface

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Introduction

Almost half (44%) of all child deaths occur in the neonatal period, with a significant part during the first days of life [1]. Globally, early-onset neonatal sepsis accounts for about 15% of neonatal mortality [1-3]. The risk of rapid deterioration necessitates early diagnosis and therapy, but clinical symptoms are nonspecific or can even be absent. Currently available laboratory tests such as C-Reactive Protein (CRP) are not sufficiently sensitive to decide whether or not to initiate antibiotic treatment [4]. Problems in identifying children at risk for early-onset sepsis result in repetitive blood sampling, prolonged and unnecessary antibiotic therapy and finally in resistant microorganisms and high costs for global health care systems. Methods to non-invasively identify newborns in danger for early-onset sepsis would therefore be very important.

Microcirculatory dysfunction is a key factor in the pathophysiology of sepsis and septic shock [5]. Different research groups could demonstrate severe changes in the quality of blood flow both in animal models of sepsis and in septic humans. Early alteration of microcirculatory blood flow was associated with poor outcome [6,7]. A time-dependent significant decrease in perfused vessels and microvascular flow index during a hypodynamic state of sepsis could be demonstrated in a pig model [8]. Moreover, it has been shown that microcirculatory dysfunction correlates with markers of endothelial activation in children suffering from meningococcal disease [9].

However, little is known about the microcirculation in newborns at the very onset of infection before severe symptoms develop. In the absence of hypotension adult patients do not show measureable microcirculatory flow abnormalities at the beginning of sepsis [10].

Our group previously found changes in microcirculatory flow in infection in preterm and term infants [11,12]. But prospectively measuring the microcirculation with Sidestream Dark Field imaging (SDF imaging) as a screening tool for early onset neonatal infection has not been attempted. For this goal an easy bedside scoring system would be necessary to succeed in identifying an infection early in the disease course.

Methods

Patient recruitment

In this prospective study, we consecutively included every term newborn born between March 1st to April 30th 2011 on our maternity unit with 37 to 42 weeks of gestation, birth weight above 2000

grams and written parental consent. Exclusion criteria were significant congenital malformation, drug abuse and dark skin pigmentation-due to technical limitations in visualizing the microcirculation with SDF imaging in this population. The study was approved by the ethics advisory board of the medical faculty.

On our ward, infants with risk factors for infection (maternal fever over 38°C, elevated maternal CRP, meconium stained amniotic fluid, fetal tachycardia, premature rupture of membranes (>18 hours) and clinical signs of infection) are clinically monitored with physical exams and receive routine CRP measurements from cord blood and at 36-48 hours together with the neonatal screening. More frequent laboratory examinations and treatment decisions are up to the attending physician's discretion. Physicians and nurses were unaware of the results of the microcirculation measurements and treatment was independent from the study.

Microcirculatory measurement

The microcirculation was assessed once during the first 72 hours of life at the upper ear conch with the "MicroScan" microscope (Micro Vision Medical Amsterdam, The Netherlands) using the Sidestream Dark Field (SDF) imaging technology [13-15].

SDF imaging visualizes the microcirculation by means of monochromatic green light with a wavelength of 530 nm, which is emitted through LEDs that are placed concentrically around an optical fiber. The hemoglobin of the erythrocytes absorbs this light while other structures reflect it. Thus, perfused vessels can be seen in negative contrast in the skin up to a depth of 3 mm. The microcirculation is directly visualized on a PC as a real-time video sequence with a resolution of one pixel per µm [16-20]. For every infant we generated three video sequences of the microcirculation of 10 second duration each. The investigator was blinded towards the clinical risk factors and health status of the child. Heart rate, oxygen saturation, age in hours, gestational age, temperature, actual weight, birth weight and mode of delivery were prospectively recorded. Additional data-which could bias the investigator-such as risk factors for infection, medical history and treatment were obtained retrospectively from the medical records.

Microcirculatory analysis

After enrolment of all newborns, stored video sequences were blinded and analyzed offline with the standard Automated Vascular Analysis program (AVA 3.0, Micro Vision Medical Amsterdam, The Netherlands) by one investigator. The evaluation in our study was performed as suggested by De Backer et al. [21]. The AVA program offers a semi-automatic analysis of functional vessel density, vessel surface and diameter distribution by automatically detecting vessels on the video. Functional vessel density accounts for the cumulative length of all vessels per field of view in mm/mm^2. In the AVA program this parameter is also defined as Total Vessel Density (TVD). The diameter distribution is defined as the percentage of total vessel length of small (<10 µm), medium (10-20 µm) and large vessels (>20 µm). Vessel surface stands for the percentage of the image area, which is covered by vessels in percent (%) and therefore combines the parameters diameter distribution and vessel length. The analyzer can correct for artifacts such as hair and dander.

Additionally, we assessed the quality of flow Microvascular Flow Index (MFI) by attributing different flow qualities to each quadrant of the image. Flow qualities were defined as 0=no flow, 1=intermittent, 2=sluggish, 3=continuous (normal) and 4=hyperdynamic [21]. The parameter hyperdynamic flow was newly introduced by the AVA program and is therefore rarely used in literature. The MFI is calculated as mean of the flow qualities of all quadrants for small, medium and large vessels.

To establish a real-time bedside evaluation, we introduced a bedside score, which was directly determined during the video recordings and was therefore not blinded (except for the fact that the examiner had no information on the clinical status of the child). For faster and easier quantification, we scored the general quality of flow in the total image area (in contrast to the AVA score, which is determined per quadrant). We assigned a score of 0 for normal flow, a score of 1 for a flow, which was suspicious or the analyzer was not sure, and a score of 2 for an abnormal flow. Suspicious flow was defined as slight flow abnormalities such as either mildly sluggish, intermittent or hyperdynamic flow. Abnormal flow was classified as severely impaired flow such as no flow, severely intermittent, sluggish or hyperdynamic flow. Out of three video sequences we generated the mean, which was described as bedside score in this study. For comparison, we also assessed the standard MFI using AVA (as described above) directly at the bedside in addition to later blinded off-line analysis. Figure 1 depicts a measurement of the microcirculation and analysis obtained from the videos.

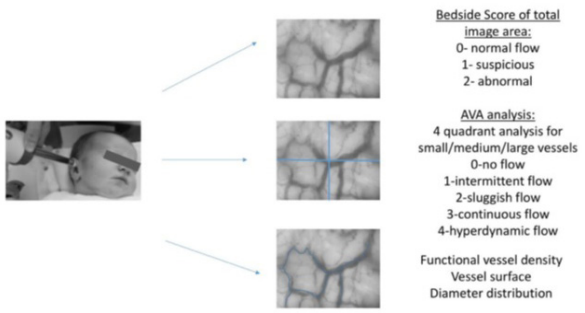


Figure 1: Measurement and analysis of the microcirculation.

After the end of the enrolment period, the data of infants was assigned to either the Antibiotic group (group A), which consisted of children that were treated for newborn infection with a CRP value above 0.5 mg/dl, or the control group (group C) that was composed of healthy children without antibiotic treatment and CRP values of less than 0.5 mg/dl or no CRP measurement.

Statistics

For statistical analysis Graph Pad Prism 7.0 (La Jolla, USA) was used. Data are presented as mean values of the three sequences with a confidence interval of 95%. We analyzed nonparametric data with Mann-Whitney-U-Test and parametric data with unpaired t-test. Moreover, non-linear regression analysis was used to analyze the effects of time points on different analysis values. ROC analyses have been performed for the results of the offline- MFI and the bedside scores.

Results

Study population

Out of 158 consecutively live births, 110 neonates were prospectively enrolled into the study and SDF imaging was obtained during

the first 72 hours of life. 48 patients were excluded due to missing parental consent (13 cases), hospital transfer (12 cases), prematurity (10 cases), skin pigmentation in African neonates (11 cases) and early discharge (two cases). None of the children assessed were transferred to intensive care.

13 children received antibiotic treatment (Group A) due to suspected neonatal infection, leaving 95 children as healthy control group (Group C). In our study population, this accounts for an overall infection rate of 11.8%. Two children were excluded from the study after enrolment, as one mother had poly-drug abuse during pregnancy-which was not known at enrolment-and one child treated with antibiotics despite persistently negative CRP did not match our inclusion criteria of group A or C. An overview of our study population is depicted in figure 2.

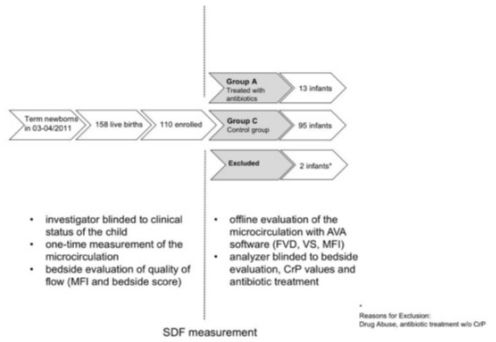


Figure 2: Study population.

Mean CRP in group A was 2.7 mg/dl [95% CI: 2.0 - 3.5] in contrast to a CRP value of 0.21 mg/dl in group C [95% CI: 0.1 - 0.3], which was measured in 44 out of 95 children (46%). All blood cultures remained negative except for one case of *Staphylococcus hominis*, probably a contamination. Seven children were positive for coagulase negative *Staphylococcae,* one for *Escherichia coli*, one for *Streptococcus agalactiae* in the swab of the ear and one child was positive for *Candida albicans* in the gastric aspirate (all patients of group A).

The retrospective analysis revealed that SDF scans in group C were obtained significantly earlier than in group A (group C: 26 hours of life [95% CI: 24-29], group A at 37 hours of life [95% CI: 29-45] p <0.05). The clinical data of the two groups such as birth weight and mode of delivery (Table 1) did not differ.

Changes in microcirculatory parameters

Data on microcirculatory parameters in Group A and C are presented in table 2. Functional vessel density (FVD) was significantly lower in group A in comparison to C (p<0.05). The decrease of Vessel Surface (VS) in group A did not reach significance. Most importantly, we observed significant differences in the quality of flow between groups A and C. The mean bedside score of group A was significantly higher than in group C (p<0.0001) (Table 2). The MFI obtained at the bedside as well as in the blinded offline analysis showed good agreement and confirmed the result of the bedside score. In all analysis, bedside and off-line neonates of the group A had more hyperdynamic flow. The bedside score could distinguish the two groups with barely an overlap. The quality of flow of the off-line MFI and of the bedside score were additionally analyzed with ROC analyses (Table 3). For the off-line MFI, the area under the curve was 0.78 with a p-value of 0.001 (Figure 3). For the bedside score the ROC analysis showed an area under the curve of 0.85 (Figure 4) with a p value <0.0001, indicating a sensitivity of 77% and specificity of 86% for an infection with a bedside score above 0.85 and a positive likelihood ratio of 5.6.

	Antibiotic Group (A) n=13	Control Group (C) n=95	p-value
Gestational age (weeks)	40.6 [40-41]	39.5 [39-40]	
Birth weight (g)	3584 [3311 - 3856]	3390 [3302-3479]	
Male sex (%)	53%	52%	
Apgar 10 minutes	10 [9.7-10]	10 [9.7-10]	
Umbilical artery pH	7.3 [7.2-7.3]	7.3 [7.3-7.3]	
Saturation (SaO2%)	98 [97-99]	98 [98-98]	
Heart rate (Min)	122 [113-131]	117 [114-119]	
Axillary temperature at time of measurement (°C)	37 [36.8-37.3]	37 [37-37]	
Age at the measurement (h)	37.3 [29.3-45.3]	26.4 [23.6-29.3]	p<0.001

Table 1: Clinical data of group A and C.

The clinical data of the two groups is presented as mean and 95% confidence interval. There were no significant differences between the groups except for the hour of life at the microcirculatory measurement, which was significantly later in the Antibiotic group (unpaired t-test/Mann-Whitney test).

	Antibiotic Group (A) n=13	**Control Group (C) n=95**	**p-value**
Functional vessel density (mm/mm²)	14.0 [13.6-14.3]	14.5 [14.3-14.8]	p<0.05
Vessel surface (mm2/mm2) × 100%	24.7 [23.3-26.1]	25.9 [25.4-26.4]	n.s.
Diameter distribution (%):			
• Small vessels (0-10 µm)	52 [51-53]	53 [49-57]	n.s.
• Medium vessels (10-20 µm)	30 [29-31]	29 [26-32]	
• Large vessels (> 20 µm)	18 [17-19]	18 [15-20]	
Microvascular Flow Index (MFI) at the Bedside	3.2 [2.9-3.5]	3.0 [2.9-3.0]	p<0.001
Microvascular Flow Index (MFI) off-line (Blinded)	3.2 [2.9-3.5]	3.1 [3.0-3.1]	p<0.001
Bedside score	1.2 [0.8-1.7]	0.3 [0.2-0.4]	p<0.0001

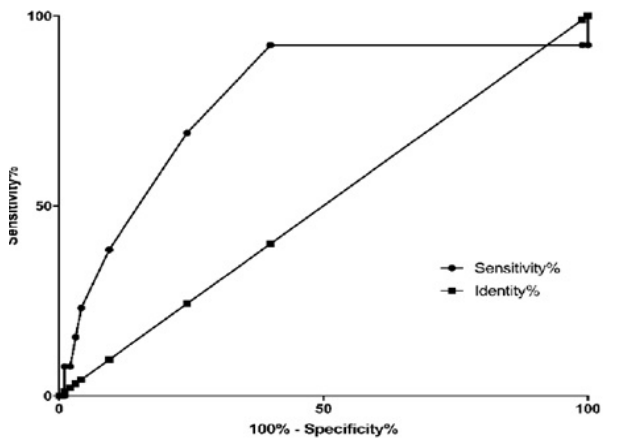
Table 2: Microcirculatory data of group A and C.

The microcirculatory variables differ significantly between both groups (Unpaired t-test/Mann-Whitney test).

	Bedside Score Cutoff 0.85	**Off-line Mfi Cutoff 3.15**
Sensitivity (%)	77	69
Specificity (%)	86	75
Positive predictive value (%)	44	28
Negative predictive value (%)	97	95
Likelihood ratio for infection	5.6	2.9

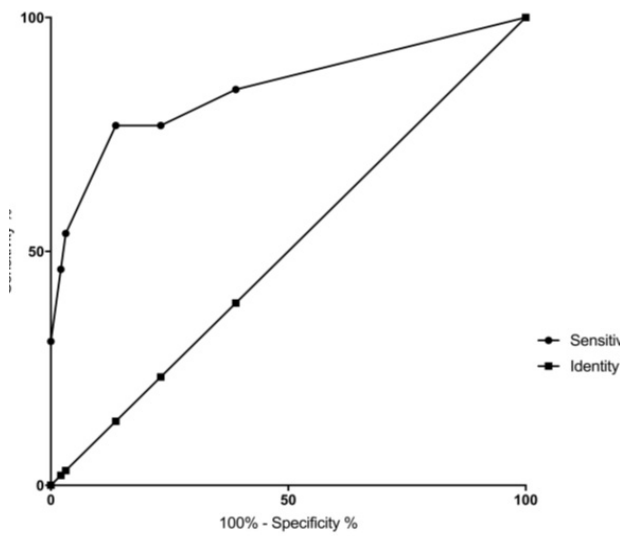
Table 3: Comparison of the bedside score and the off-line MFI.

The bedside score shows a higher sensitivity and specificity than the off-line MFI.



	Results
Area under the ROC curve	
Area	0.781
Std. Error	0.07414
95% confidence interval	0.6357 to 0.9263
P value	0.0011

Figure 3: ROC analysis of the off-line MFI score.



	Results
Area under the ROC curve	
Area	0.8462
Std. Error	0.07209
95% confidence interval	0.7049 to 0.9874
P value	<0.0001

Figure 4: ROC analysis of the bedside score.

A regression analysis was used to exclude a potential bias due to a later measurement time point in the group A. R^2 was calculated to be <0.01 for all regression analysis indicating that the time point does not predict either bedside score, MFI, VS or FVD.

Discussion

Our study demonstrates that characteristic changes in the microcirculation can be detected very early in newborn infection before clinical deterioration and therefore might be suitable screening parameters on newborn wards. Functional Vessel Density (FVD) is reduced and flow changes to hyperdynamic very early in infection. Alterations in flow were identified immediately at the bedside, as well as in the later off-line standard AVA analysis.

A central function of the microcirculation is delivery of oxygen to the periphery. We could show that functional vessel density is reduced early on in infection. This finding is consistent with previous studies demonstrating lower functional vessel density in patients with infection and sepsis [7,12,13,16,19]. The FVD is well validated and seems to be one of the best quantitative indicators of microvascular perfusion. As only RBC-perfused microvessels are included in its measurement, FVD is an indirect parameter for the oxygen transport and distribution capacity [22]. In severe infection, microcirculatory flow is redistributed to maintain oxygen delivery to the central organs. In several studies, sublingual microcirculatory abnormalities have been described and linked to morbidity and mortality in septic shock patients [6,7,19,23]. In this context, a significantly diminished perfusion of small sublingual capillaries in adult patients with septic shock increased over time in survivors in comparison with non-survivors [13]. Similar results were found in patients of pediatric intensive care units with persistent low microcirculatory vessel density in non-survivors of sepsis [7]. Severe abnormalities in microvascular perfused vessel density were associated with organ dysfunction and mortality [24]. In our study population, we could demonstrate a decrease in FVD already early in patients with still mild infection. Nevertheless, analyzing FVD is a very time consuming method, therefore we were searching for other parameters to screen for newborn infection.

In neonates, the microcirculation of the skin reacts early in any disease process, due to the high skin surface ratio and the limited ability to increase the already high heart rate further to increase cardiac output. This might to some extent explain the specific finding of hyperdynamic microcirculatory flow in our study. In adults, sepsis leads to either very heterogeneous or sluggish or no flow [13,19]. By contrast, we found that neonates with mild to moderate infection have rarely the sluggish flow described for severely septic adult patients, but instead demonstrate increased flow. Another explanation for this apparent discrepancy might also be the very early stage of infection in our study in opposite to the septic shock in the microcirculation studies of adults. Like in adults, sluggish to no flow can be observed in pediatric patients in shock [7]. None of the neonates included in our study needed systemic treatment for arterial hypotension, organ dysfunction etc. as seen in severe sepsis. Therefore, we suggest that hyperdynamic flow might be present as an early finding in infection proceeding to severe microcirculatory dysfunction seen in sepsis and septic shock.

As the ROC analysis showed a likelihood ratio of 2.9 for infection for hyperdynamic flow (MFI>3.15) or even 5.6 for a bedside score above 0.85. Thus, these could be suitable parameters for non-invasive monitoring of term infants at risk for postnatal infection. The negative predictive value of 95% for a MFI above 3.15 or even 97% for a bedside score of above 0.85 underlines that analyzing the quality of flow could be a very sensitive screening parameter in future.

Since the scoring system of the MFI was originally developed with adult data, in which most analyzed changes are sluggish to no-flow, abnormal results with mainly hyperdynamic flow might be underestimated. As, the AVA-software assigns 3 points to normal flow, 4 to hyperdynamic and 2-0 to sluggish or no flow, scans with

heterogeneous perfusion combining sluggish and hyperdynamic flow in one image can be scored at a mean normal value. Even though in our study most of our data showed a purely hyperdynamic flow, this could be different when studying children with more severe infection. Moreover, a scoring system between 0 to 4 for all quadrants is quite time consuming and needs a lot of expertise and can therefore vary between observers. To address this issue, we introduced another scoring system in which we scored only normal (=0), suspicious (=1) or abnormal flow (=2) irrespective of the actual quality of the flow. Furthermore, only one score was assigned to the whole image. Our results show that this method can be easily applied at the bedside and might be even more effective in diagnosing mild infection in newborns as it could discriminate very well between both groups. For a screening parameter, it would be enough to identify pathological flow no matter whether it is hyper-or hypodynamic, which is less time consuming and might even be less subjective. Recently, it was demonstrated that the blood flow was reliably scored in adult intensive care patients at the bedside by the responsible nurses [25]. The easy and observer-independent application along with little time expenditure are essential requirements for the successful implementation of a screening tool into the clinical routine.

Our study has some limitations. One potential bias in our study group could be that measurements in group A were obtained at a later time point compared to group C. The difference in time point for group A is potentially due to the smaller size of the group. In group A two out of 13 neonates received microcirculatory measurements on the third day of life, which was within our defined prospective study range, but shifted the mean time point of the study. As the observer was blinded to the clinical data of the children, this did not influence the result and we found no correlation between time point of measurements and microcirculation data. However, due to the small number of children analyzed in group A, it was not possible to perform sub-analyses on the microcirculatory parameters for different time frames. It would be desirable to repeat the study with a larger number of infants and shorter time-frame to fully rule out a bias due to differences in the time point.

All children of group A were treated with antibiotics for infection. It cannot be ruled out that this treatment has an effect on the microcirculation, however, this has yet to be studied. Another limitation is the size of the study group. It would be desirable to have a look at a larger study population. However, this is the largest study of newborn infection and microcirculatory data published up to date. We are planning a larger study with shorter time frames to verify the results shown in this paper.

Another problem encountered is that the method cannot visualize the microcirculation of neonates with dark skin pigmentation, as the melanin spots in the skin reflect the LED-light. Sublingual measurements used in adults are difficult due to the strong suck reflex in this age group. However, obtaining images successfully from the buccal mucosa has been reported [26]. In our population, the number of children with dark skin is very low, so we do not think that this has influenced the results of our study.

Conclusion

All together our study has important findings, which deserve to be addressed in further studies. First, SDF imaging is a simple non-invasive method to evaluate the microcirculation and therefore could be a cost-effective screening method for identifying neonates at risk for infection. Second, in neonates with mild infection hyperdynamic flow seems to be a major pathophysiological finding. Neonates with infection show altered flow and a reduced functional vessel density very early in the disease course. These changes can be easily detected at the bedside and could thus prove to be useful parameters to screen for neonatal infections.

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Appendix 1

Clinical Data of Group A and of Children Excluded from the Study

	Hours of Life (h) at Measurement	Max CRP (mg/dl) before Measurement	Max CRP (mg/dl) after Measurement	Antiinfective Treatment	Exclusion Criteria
011 NG	57	3,48	1,14	Ampicillin, Cefotaxim, Nystatin	
023 NG	23	2,46	1,89	Ampicillin, Cefotaxim, Nystatin	
033 NG	32	1,62	0,86	Ampicillin, Cefotaxim, Nystatin	
061 NG*	7	0,18	8,99	Ampicillin, Cefotaxim, Tobramycin Nystatin	*Excluded from study due to polydrug abuse during pregnancy
062 NG	41	4,93	3,14	Ampicillin, Cefotaxim, Nystatin	
069 NG	23	1,61	1,93	Ampicillin, Cefotaxim, Nystatin	
076 NG	28	0,89	0,48	Ampicillin, Cefotaxim, Nystatin	
077 NG*	35	0	0,18		*Excluded from study due to negative CRP values and no antibiotic treatment despite admission to ICU, therefore it was not included to group C.
089 NG	49	2,72	0,67	Ampicillin, Cefotaxim, Nystatin	
094 NG	33	5,04	3,15	Ampicillin, Cefotaxim, Nystatin	
096 NG	32	3,98	1,98	Ampicillin, Cefotaxim, Nystatin	
102 NG	44	2,09	1,96	Ampicillin, Cefotaxim, Nystatin	
103 NG	57	2,5	0,47	Ampicillin, Cefotaxim, Nystatin	
106 NG	17	1,46	1,04	Ampicillin, Cefotaxim, Nystatin	
110 NG	49	2,84	0,61	Ampicillin, Cefotaxim, Nystatin	

The clinical data of group A and of children excluded from the study is presented in the Appendix. All children of group A were positive for infection. 2 children were excluded from the study. All children from group A were treated with antiinfectives.