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ORIGINAL

Leukocytes influence peripheral tissue oxygenation and perfusion in neonates

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ABSTRACT

Background. Leukocyte counts may influence peripheral (micro) circulation due to changes in rheology. The aim of this study was to investigate a possible association between leukocyte counts and peripheral tissue oxygenation/perfusion measured with near infrared spectroscopy (NIRS) in term and preterm neonates.

Methods. In this observational study we included term and preterm neonates within the first 2 months of life, in whom peripheral tissue NIRS measurements were performed and blood samples (leukocytes and C reactive protein (CRP)) taken to investigate clinical signs of infection. Tissue-oxygenation index (TOI), fractional oxygen extraction (FTEO), oxygen delivery (DO_2), oxygen consumption (VO_2) and vascular resistance (VR) were measured by NIRS and venous occlusion method. TOI, FTOE, DO_2, VO_2 and VR were correlated to leukocyte counts on the same day and maximal CRP levels within 24 hours (CRP max).

Results. In 180 infants, with a mean gestational age of 35.5±3.3 weeks, leukocyte counts were 16546± 8830/µl (median 14830; range 1790 to 67840) and CRP max was 8.0± 19.0 mg/l (median 0.0; range 0.0 to 110.0mg/l).

TOI was 71.1±5.5%, FTOE 28.5±6.1%, DO_2 46.7±19.7, VO_2 12.5±4.4 and VR 11.7±6.4.

Leukocyte counts correlated negatively (r= -0.21; p= 0.005) with TOI and positively (r=0.17; p=0.029) with VR. Correlations with CRP max did not reach significance.

Conclusion. We demonstrated that peripheral tissue oxygen consumption decreases and vascular resistance increases with increasing leukocyte counts.

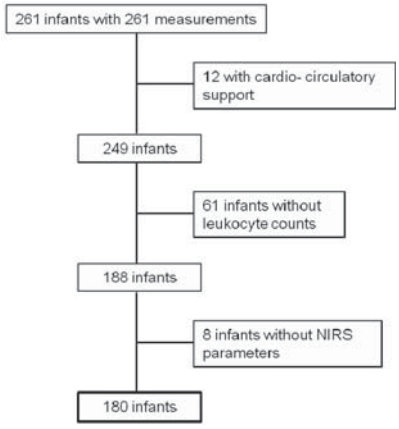
Key words: near-infrared spectroscopy, neonate, microcirculation, leukocytes.

Introduction

Peripheral (micro) circulation is influenced by changes in rheology due to changes in blood cell counts, (1,2) and by cardiovascular (3) and inflammatory processes. (4,5) Symptoms of inflammatory processes, such as hyperthermia, lethargy, tachypnea or poor crying are non-specific in neonates. (6) Therefore, when infection is suspected, in addition to these clinical signs, laboratory parameters may be helpful. C reactive protein (CRP) levels and leukocyte counts are two of the most widely used parameters in routine clinical diagnosis of inflammation and infection. (6,7)

To measure changes in peripheral (micro) circulation and oxygenation non-invasively, different devices can be used. (8) Near infrared spectroscopy (NIRS) is one of them, which enables continuous measurement of tissue oxygenation and perfusion. (9,10) Previous studies have described associations between inflammatory processes and peripheral muscle oxygenation in adults. (11-13) Recently, our study group was able to show differences in peripheral oxygenation and perfusion in neonates with CRP elevation. (14)

The aim of this study was to demonstrate a possible association between varying leukocyte counts and peripheral tissue oxygenation/perfusion measured with NIRS in term and preterm neonates. We hypothesized that peripheral oxygenation and perfusion would be impaired in association with increasing leukocyte counts.



NIRS, near infrared spectroscopy.

Figure 1. Numbers of infants, who were eligible, excluded and included for the study.

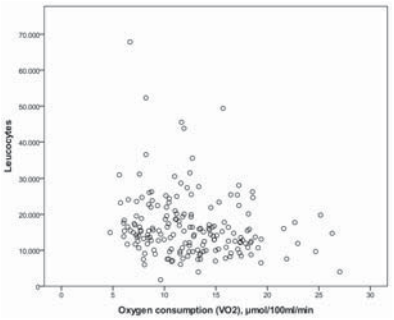


Figure 2. Correlation between leukocytes and peripheral oxygen consumption (VO2) in 180 infants (r = -0.21; p = 0.005).

Methods

Patients

This observational study was conducted at the Medical University of Graz, Division of Neonatology. We included term and preterm neonates within the first 2 months of life in whom peripheral tissue NIRS measurements were performed and blood samples were taken to investigate clinical signs of infection. We excluded neonates with congenital malformations or any cardio-circulatory support. No other specific entry criteria were defined.

Neonates born between January 2006 and February 2011, who satisfied the above criteria, were included.

NIRS measurements were approved by the local ethics committee. Informed consent was obtained from the parents before measurements.

NIRS

Peripheral muscle oxygenation and circulation were measured by NIRS with the venous occlusion method.

NIRS measurements were carried out with the NIRO 300 (Hamamatsu Photonics, Japan). The optodes were attached to the lateral side of the left calf, the inter-optode distance was 3.0cm, and the sampling rate was 2/sec. A differential path length factor of 5.51 was used. (15) The spatially resolved method (NIRO 300) enables non-invasive continuous measurement of the tissue oxygenation index (TOI) and of changes in the concentration of oxygenated haemoglobin (HbO_2) and deoxygenated haemoglobin (Hb). Changes in the concentration of total haemoglobin (Hb_{tot}) were calculated from the sum of changes in HbO_2 and Hb.

Venous occlusion

Venous occlusion was performed using a pneumatic cuff placed around the thigh. Venous occlusion causes an increase in calf blood volume by undisturbed calf arterial (in) flow and interrupted venous (out) flow. Changes in HbO_2, Hb and Hb_{tot} during venous occlusion are only caused by arterial inflow and oxygen consumption of the tissue.

Protocol

A detailed protocol of the measurement and the calculations of the NIRS parameters have been reported in a previous study. (16)

Blood samples

NIRS measurements were performed after routine blood samples were taken to investigate clinical sign/s of infection. Blood cell counts and CRP levels were measured. In neonates with persisting signs of infection a second blood sample was taken within 24 hours of the NIRS measurement.

Statistical analysis

Only data from measurements passing published quality criteria were analysed. (16) The Spearman's rank correlation coefficients were used to assess the correlations between NIRS parameters (tissue oxygenation index, fractional oxygen extraction, oxygen consumption, oxygen delivery, vascular resistance) and leukocyte counts. In addition we calculated the correlation between NIRS parameters and maximal CRP levels (CRP max), whereby in neonates with CRP max < 0.6mg/l the CRP max was set to zero. P-values of < 0.05 were regarded as statistically significant and of < 0.01 as highly significant. The statistical analysis was performed using the statistical software SPSS 19.0 (SPSS Inc, Chicago, Ill).

Results

From January 2006 to February 2011, 261 infants were included in the study and 180 neonates were analysed (figure 1). Demographic and clinical data of the 180 analysed infants are presented in table 1. Data of peripheral muscle oxygenation and perfusion are shown in table 2.

Leukocyte counts were $16546 \pm 8830/\mu$l (median 14830; range 1790 to 67840). Leukocyte counts correlated negatively ($r = -0.21$; $p = 0.005$) with tissue oxygen consumption (VO_2) (figure 2) and positively ($r = 0.17$; $p = 0.029$) with vascular resistance (VR).

CRP max was 8.0 ± 19.0 mg/l (median 0.0; range 0.0 to 110.0 mg/l). In 114 infants no CRP elevation (CRP $<$ 0.6mg/l) was measured.

Taking all neonates into account, there was a trend that CRP max correlated negatively with TOI ($p = 0.068$) and with VO_2 ($p = 0.096$), without reaching significance.

Discussion

To our knowledge this is the first study which showed an association between leukocyte counts and peripheral muscle oxygenation and perfusion in term and preterm neonates. We demonstrated that peripheral tissue oxygen consumption decreases and vascular resistance increases with increasing leukocyte counts.

These results suggest alterations in peripheral microcirculation with increasing leukocyte counts, which could be explained by changes in rheology primarily caused by increased leukocyte

Table 1. Demographic and clinical parameters of the study group.

	n	Mean	SD	Median	Range
Gestational age (wk)	180	35.5	3.3	35.1	24.4-42.0
Birthweight (g)	179	2545	847	2400	700-5400
Age on day of measurement (h)	180	53	146	22	0-1392
Actual weight (g)	175	2577	797	2450	760-4480
Calf diameter (cm)	177	3.0	0.5	3.0	1.7-4.2
Calf subcutaneous adipose tissue (cm)	178	0.3	0.1	0.3	0.1-0.6
APGAR 5	180	9.1	1.2	9.0	2.0-10.0
HR - heart rate (bpm)	180	129	14	128	93-175
SpO_2 – oxygen saturation (%)	180	95.9	3.1	98.2	85.3-100.0
Mean arterial blood pressure (mmHg)	167	42.3	7.4	40.0	19.5-68.0
Temperature rectal (celsius)	164	36.9	0.3	36.9	35.9-37.9
Temperature peripheral (celsius)	179	35.4	0.9	35.6	32.5-37.6
Central capillary refill time (sec)	178	3.2	0.8	3.1	1.3-7.1
Peripheral capillary refill time (sec)	178	3.1	0.6	3.0	1.4-5.3

Table 2. Peripheral muscle oxygenation and perfusion measured with near infrared spectroscopy (NIRS) in 180 infants.

	n	Mean	SD	Median	Range
Tissue oxygenation index (TOI), %	180	71.1	5.5	71.8	48.9-85.0
Fractional oxygen extraction (FOE), %	180	28.5	6.1	28.0	15.0-50.4
Oxygen delivery (DO_2), μmol/100ml/min	180	46.7	19.7	45.3	14.9-110.4
Oxygen consumption (VO_2), μmol/100ml/min	180	12.5	4.4	11.9	4.7-27.0
Vascular resistance (VR), mmHg/ml/min	157	11.7	6.4	9.9	2.8-38.6

count. Because of their large volume and low deformability (17), white blood cells can influence the microcirculatory flow even though their total volume concentration is much lower, in comparison to red blood cells. (1) The Influence of Hb on peripheral oxygenation and perfusion has already been demonstrated using NIRS in several studies. (18-20)

Increasing leukocyte counts with changes in peripheral oxygenation and perfusion might be associated with inflammatory processes. Studies have demonstrated associations between inflammatory processes and changes in the microcirculation. (4,5,21) It is known that inflammation can cause decreased deformability, accumulation and increased endothelial cell adhesion of leukocytes, which can lead to capillary plugging and organ/tissue ischemia. (22-25)

Adults with septic conditions had significant changes in VO_2 measured with NIRS. VO_2 was lower in patients with SIRS (13) and correlated negatively with inflammatory markers. (12) These findings are in accordance with our results in VO_2 in neonates.

In a previous study we demonstrated that neonates with elevated CRP levels have reduced peripheral tissue oxygenation and perfusion. (14) In the present study there were only trends but no significant correlations between CRP max and NIRS parameters. This finding is most probably due to the fact that in 114 of 180 infants CRP was negative (< 0.6mg/l). Moreover, this finding supports the hypothesis that leukocyte levels themselves are associated with changes in peripheral oxygenation and perfusion.

The present study has some shortcomings. First of all, the study population

was rather heterogeneous, and correlations were rather weak. Therefore, the clinical significance of these findings must be interpreted with caution. Nevertheless, correlations between leukocyte counts and VO_2/VR were significant, even though other parameters are known to influence peripheral oxygenation and perfusion. (18-20,26-29)

This study further contributes to the understanding of changes in oxygenation and perfusion in the peripheral tissue of newborn infants. The technique of NIRS is improving constantly and has the potential to become a useful, noninvasive diagnostic bedside tool with the ability to measure subtle disturbances in oxygenation and perfusion in peripheral tissue, before vital organs are affected, in order to initiate appropriate therapy at an earlier stage.

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