Methemoglobinemia Associated with Late-Onset Neonatal Sepsis: A Single-Center Experience

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Abstract

Objective Methemoglobinemia (MetHb) is a rare congenital or acquired cause of infantile cyanosis. We examined the role of MetHb in a neonatal intensive care unit (NICU).

Study Design A retrospective observational study was conducted reviewing blood gas analyses of hospitalized newborns over a 2-year period. MetHb-positive patients (MetHb >1.8%) were matched with a control group for gestational age, weight, disease, and illness severity at admission. Maternal, neonatal, clinical, and laboratory parameters were collected and analyzed in both groups.

Results MetHb incidence was 6%. The mean MetHb in the case group was 7.2%, and the first positive samples were observed at a mean of 22 days of life, 6 days prior to clinical or culture-proven sepsis. We identified low maternal age (31 vs. 34 years; $p = 0.038$), sepsis (90 vs. 45%; $p = 0.022$), and protracted parenteral nutrition (46 vs. 23 days; $p = 0.013$) as risk factors for MetHb, and early minimal enteral feeding as protective factor (12th vs. 9th day; $p = 0.038$).

Conclusion MetHb has a high occurrence in NICU and can be a helpful prognostic indicator of an infectious process. Understanding and prompt identification of MetHb can allow pediatricians to implement a life-saving therapy.

Keywords
- observational study
- cyanosis
- anemia
- hypoxia
- newborn

Methemoglobinemia (MetHb) is a rare but serious cause of infantile cyanosis (blue baby) characterized by abnormal levels of hemoglobin (Hb), in which the iron component of Heme is oxidized from the ferrous (Fe_{2+}) to the ferric (Fe_{3+}) state and unable to bind oxygen.[1] This leads to functional anemia and tissue hypoxia with all its subsequent clinical risks.

A physiological oxidation is rapidly compensated by antioxidant enzymatic systems such as NADH cytochrome b5-reductase (methemoglobin reductase).[2] If these self-protecting mechanisms fail, MetHb will onset.

MetHb may be congenital (due to a hereditary deficiency of methemoglobin reductase or to abnormal Hb as HbM)[3] or acquired secondary to endogenous conditions (diarrhea, acidosis)[4] or to oxidizing xenobiotics. Isolated cases of metHb associated with metoclopramide,[5] paracetamol,[6] local anesthetics (as benzocaine or lidocaine/prilocaine),[6–9] and dietary nitrite/nitrate[10] have been reported. Among the endogenous causes, in adult and pediatric patients, it has been hypothesized that sepsis can cause MetHb due to the increased production of nitric oxide (NO) stimulated by proinflammatory cytokines and bacterial lipopolysaccharides.[11,12] Therapeutic use of inhaled NO can also cause MetHb so that in newborns treated for persistent pulmonary hypertension, MetHb is periodically checked.[13]

The aim of the study was to determine the incidence of MetHb in neonatal intensive care unit (NICU) babies and to identify risk factors, other than inhaled NO, to establish which newborns admitted to the NICU should be carefully monitored.

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Materials and Methods

An Institutional Review Board approval was obtained. This retrospective observational study was conducted reviewing the clinical charts of infants admitted in the period between January 2016 and December 2017 at the Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro," University of Palermo. Blood gas analyses of all newborns admitted to the NICU during the study period were reviewed.

Patients who underwent NO therapy for pulmonary hypertension and those with a diagnosed cyanotic heart disease were excluded.

During the NICU hospitalization, MetHb values were directly reported on arterial or arterialized capillary blood gas analyses performed with ABL 800 flex (Radiometer, Copenhagen, Denmark) to closely monitor ventilatory gases and electrolyte parameters.

MetHb level $>$ 1.8% of total Hb was considered positive.

Each MetHb positive patient was matched with a control infant for gestational age, weight, disease, and neonatal illness severity at admission using the NICU risk score (Score for Neonatal Acute Physiology [SNAP] II and SNAP with Perinatal Extension [SNAPPE] II).[14]

Maternal, obstetrical, clinical, and pharmacological data were analyzed.

Neonatal clinical and laboratory parameters were collected from medical records at T_0 (48 hours prior to MetHb increase), T_1 (day of the first positive MetHb), and T_{max} (day of maximum value of MetHb). In the control group, T_1 was assessed on the 22nd day of life (corresponding to the mean of days of the first MetHb increase in the case group) and T_0 on the 20th day of life.

For each infant were recorded: feeding regimen, day of surgery, blood transfusions, pharmacological treatments (type and duration), type and onset of infections (defined as early, late, or very late onset clinically suspected or culture-proven sepsis), comorbidity, and mortality. In survivors until hospital discharge, we considered the duration of invasive mechanical ventilation, parenteral nutrition, and hospital stay.

Statistical analyses were performed using open-source statistical R 3.5.1 software (R Foundation for Statistical Computing, Vienna, Austria), with chi-square tests and Fisher's tests for categorical variables and Student's *t*-tests, Wilcoxon tests, and Kruskal–Wallis-tests for continuous variables; $p \leq 0.05$ was considered significant.

Results

During the 2-year study period, the incidence of pathological MetHb was 6% (11/182 eligible newborns), excluding patients treated with NO for pulmonary hypertension or with cyanotic heart diseases.

In the case and control groups, 91% of patients were preterm, with a mean gestational age of $32^{6/7}$ weeks (range: $24^{1/7}$–$37^{1/7}$ weeks). The mean birth weight was 2,063 g (range: 665–3,470 g). All patients were in very critical conditions at admission (SNAP/SNAPPE-II 13/25). Of the infants, 64% were admitted for gastrointestinal congenital malformations and 36% for necrotizing enterocolitis; 91% of patients underwent surgery during hospital stay. Male newborns seem to be more affected by MetHb (seven males vs. four females; $p = 0.36$). The mean MetHb in the case group was 7.2% (range: 2.3–17%). The first positive samples were observed at a mean of 22 days of life (range: 8–48 days of life) and 6 days prior to the onset of clinical or culture-proven sepsis (range: 1–8 days). The maximum concentration was found at 37 days of life (range: 22–83 days of life).

At the same gestational age, birth weight, and SNAP/SNAPPE-II score, MetHb was associated with lower maternal age at delivery (31 vs. 34 years; $p = 0.038$); no other maternal risk factors (smoking, pregravidic diseases, medicaments) nor vitamin supplementation showed statistical significance.

The average length of hospital stay was longer in MetHb patients, and four infants did not survive (mortality rate: 36%). Nonsurvivors showed higher trends in MetHb peak values (10.8 vs. 4.1%; $p = 0.34$).

Circulating MetHb was significantly higher in infants with clinical sepsis (3.8 vs. 1.7%; $p = 0.022$). The most frequent culture-proven sepsis was due to *Staphylococcus spp*. Duration of parenteral nutrition was longer in the case group (46 vs 23 days; $p = 0.013$), whereas minimal enteral feeding was tolerated earlier in the control group (12th vs. 9th day; $p = 0.038$). Pharmacological therapy, vitamin supplementation, parenteral nutrition, lipid emulsion, and transfusion did not show correlation with MetHb (►**Table 1**). Two cyanotic patients with MetHb values $>$ 15% required methylene blue treatment and subsequent high intake of antioxidant vitamins until methemoglobin normalized. The others recovered spontaneously with fluid resuscitation and specific sepsis therapies.

Discussion

MetHb is a hidden problem in NICU as evidenced by the unexpectedly high incidence rate, despite the high cutoff value of MetHb, $>$1.8%, selected to minimize inclusion of patients with transitory or accidental increase.

Infants exposed in NICU to excessive oxidizing agents are particularly susceptible to oxidative stress and consequently to the development of MetHb because the antioxidant enzyme activities in erythrocytes, such as glutathione peroxidase, catalase, and methemoglobin reductase, are reduced compared with adults.[15,16] Moreover, the still predominant fetal Hb leads to more susceptibility to oxidation. In this study, almost all newborns with MetHb were preterm, confirming the role of enzymatic immaturity in the development of pathology.

Other reported that risk factors can be concomitant clinical conditions, such as anemia, acidosis, respiratory compromise, cardiocirculatory failure, slow intestinal transit, and dysbiosis in surgical patients. These comorbidities may contribute to the further formation of superoxide

Table 1 Maternal and neonatal characteristics of MetHb patients and control group

	MetHb patients ($n = 11$)	Control group ($n = 11$)	*p*-Value
Maternal and obstetric characteristics			
Age, years	34	31	**0.038**
Pre-existing diseases, %	45	27	0.375
Smoking, %	40	50	0.809
Parity	1	2	0.306
Cesarean delivery, %	72	63	0.647
Neonatal characteristics			
Apgar at 1 minute	5	7	0.573
Apgar at 5 minutes	8	9	0.282
Oxidant drugs, %	45	25	0.361
Transfusion, %	30	22	0.701
Parenteral nutrition, days	46	23	**0.013**
Intravenous lipids, %	50	50	1
Minimal enteral feeding, day	12	9	**0.038**
Colonization, %	72	60	0.536
Sepsis, %	90	45	**0.022**
Associated syndromic malformations, %	54	36	0.391
Noncyanotic heart defects, %	12	43	0.184
Hospital stay, days	46	30.5	0.066
Mortality, %	64	70	0.757

Abbreviation: MetHb, methemoglobinemia.
Note: Significant differences are in given bold.

radicals, hydrogen peroxide, or NO, which lead to methemoglobin overproduction.[2]

Our patients with MetHb showed a high rate of clinical or culture-proven sepsis. In adult and children, this association has been described, particularly in patients with diarrhea[4] and gastrointestinal inflammation,[17] independent of accidental exposure to nitrate-contaminated water or food.[18] Few studies report an association between MetHb and *Staphylococcus aureus* infection.[19,20]

In sepsis, large amounts of NO are released by activated endothelial cells. NO interacts with Hb, forming methemoglobin and nitrate (NO_3).

These nitrates (NO_3) can be transformed into nitrite (NO_2) and subsequently to other NO in the presence of nitrate reductase producing bacteria, initiating a vicious circle of severe MetHb.[2,21]

In our population, the sepsis-related MetHb might interact with dysbiosis in the gut microbiota due to delayed enteral nutrition in susceptible surgical patients.

Infectious etiology is also supported by the fact that no other exogenous sources of oxidative stress have been identified and that the medical treatment of similar pathologies was homogeneous in both groups. Furthermore, the first increase of MetHb was not correlated to local or systemic anesthetics since it occurred without temporal correlation with surgical interventions. However, the use of a local anesthetic cream (lidocaine/prilocaine) was strictly limited to term newborns in both groups.

Despite the high incidence of MetHb observed in our study, it seems to be an underdiagnosed and not considered pathological condition in the NICU because poorly symptomatic at levels below 10% and with unspecific clinical manifestations at higher levels. In modern blood gas analyzers, MetHb can be easily detected and monitored. We can suppose that some cases of MetHb secondary to specific diseases remain undetected because they rapidly recover by treating the underlying illness.

The limits of this study are the small case group and the retrospective design. However, the statistical analyses can be considered reliable, thanks to the rigorous matching protocol that reduce biases.

A multicenter prospective study is warranted to further analyze the time of onset and the progression and resolution of MetHb in relation to the identified risk factors (low maternal age, prematurity, *Staphylococcus*-related late-onset sepsis, and protracted parenteral nutrition). This would allow to confirm not only if MetHb has high frequency in NICU but also if it is a prognostic factor for sepsis.

Conclusion

As described in older children and adults, even in newborns, MetHb can be an indicator of an infectious process linked to nitrate-reducing bacteria.

It is important for the pediatrician to take MetHb into account in the differential diagnosis of cyanosis and hypoxia. The understanding of the potential etiology and the prompt identification of the MetHb can allow to implement a potentially life-saving therapy.

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Conflict of Interest
None declared.

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