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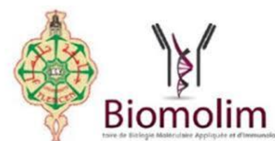

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THESIS

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Title

Immuno-pathophysiological role of miR-23b and ROS in sepsis in newborns

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Abstract

Background: Neonatal sepsis represent a major challenge in public health, where is the third most common cause of death of newborns. Neonatal sepsis characterized by misunderstood either at the molecular or at the cellular level. Here, in our study, we investigated the role of miRNA-23b in neonatal sepsis, and we studied the effect of reactive oxygen species in the activity of endothelial cells in sepsis.

Methods: Our study has two main parts. A prospective study based on Fifty-four newborns suspected sepsis. By RT-qPCR, we quantified the level of miRNA-23b in hemoculture.

Via an *ex-vivo* model, we used the umbilical cord, which's divided into four groups. Where the exposure to oxidative stress caused by $CuSO_4$ and sepsis by *Staphylococcus aureus*. After 1 hour of incubation, we isolated endothelial cells and studied them.

Results: our study showing the relationship between sepsis and miRNA-23b, where We have shown that miRNA-23b levels increased in premature and full-term newborns in the case of EOS ($p < 0.001$ and $p < 0.005$ respectively), but decreased in LOS ($p < 0.005$). And proved the negative correlation between newborns who died from sepsis and miRNA-23b level.

In the *ex-vivo* model, we have shown endothelial cells behaved differently against bacteria in the four conditions With betamethasone and without CuSO4, we noted the translocation of bacteria to the blood with a decrease in the level of miR-23b. Injection of CuSO4 into the blood induces a change in the activity of ECs and neutralizes the level of miR-23b, contribute to the defense against the translocation of bacteria in the blood.

Conclusions: The decrease in miRNA-23b levels would undoubtedly be an important factor favoring the development of neonatal sepsis. In addition to the protective role of copper-induced oxidative stress, activated endothelial cells promoting the pro-inflammatory response in the blood may help prevent the translocation of S. aureus to the blood.

Key words: Neonatal Sepsis, Endothelial Cells, Reactive Oxygen Species (ROS), miRNA-23b.

Résumé

Introduction : La septicémie néonatale représente un problème majeur de santé publique où elle est la troisième cause de décès chez les nouveau-nés. Septicémie néonatale caractérisée par une incompréhension que ce soit au niveau moléculaire ou cellulaire. Ici, dans notre étude, nous avons étudié le rôle du miARN-23b dans la septicémie néonatale, et nous avons étudié l'effet des espèces réactives de l'oxygène dans l'activité des cellules endothéliales dans la septicémie néonatale.

Matériel et Méthodes : Notre étude comporte deux parties principales. Une étude prospective basée sur Cinquante-quatre nouveau-nés suspectés de septicémie. Par RT-qPCR, nous avons quantifié le niveau de miRNA-23b en hémoculture.

Via un modèle *ex-vivo*, nous avons utilisé le cordon ombilical, qui est divisé en quatre groupes. Où L'exposition au stress oxydatif causé par le $CuSO_4$ et la septicémie par *Staphylococcus aureus*. Après 1 heure d'incubation, nous avons isolé les cellules endothéliales et les avons étudiées.

Résultats : notre étude montrant la relation entre la septicémie et le miRNA-23b, où nous avons montré que les niveaux de miRNA-23b augmentaient chez les nouveau-nés prématurés et à terme dans le cas de septicémie précoce ($p < 0{,}001$ et $p < 0{,}005$ respectivement), mais a diminué en cas de septicémie tardif ($p < 0{,}005$). Et a prouvé la corrélation négative entre les nouveau-nés décédés par septicémie et le niveau de miRNA-23b.

Dans le modèle *ex-vivo*, nous avons montré que les cellules endothéliales se comportaient différemment contre les bactéries dans les quatre conditions. Avec betaméthasone et sans $CuSO_4$, nous avons noté la translocation de bactéries au sang avec une diminution du taux de miR-23b. L'injection de $CuSO_4$ dans le sang induit une modification de l'activité des EC et neutralise le taux de miR-23b, contribue à la défense contre le Translocation de bactéries au sang.

Conclusions : La diminution des niveaux de miARN-23b serait sans aucun doute un facteur important favorisant le développement de la septicémie néonatale. En plus du rôle protecteur du stress oxydatif induit par le cuivre, les cellules endothéliales activées favorisant la réponse proinflammatoire dans le sang peuvent aider à prévenir la translocation de *S. aureus* au sang.

Mots clés : Septicémie Néonatale, Cellules Endothéliales, Espèces Réactives de l'Oxygène (ROS), miRNA-23b.

الملخص

المقدمة: يمثل الإنتان الوليدي مشكلة صحية عامة كبيرة حيث يعتبر السبب الرئيسي الثالث للوفاة عند الأطفال حديثي الولادة. يتميز الإنتان الوليدي بعدم الفهم سواء على المستوى الجزيئي أو الخلوي. هنا في دراستنا، قمنا بالتحقيق في دور ميرنا 23 ب في تعفن الدم الوليدي، ودرسنا تأثير أنواع الأكسجين التفاعلية على نشاط الخلايا البطانية في تعفن الدم حديثي الولادة.

الطريقة: دراستنا تتكون من جزأين رئيسيين. دراسة مستقبلية على أساس أربعة وخمسين مولودًا يشتبه في إصابتهم بالإنتان. بواسطة RT-qPCR، قمنا بتحديد مستوى ميرنا23ب في سائل زراعة الدم.

باستخدام نموذج خارج الجسم الحي، استخدمنا الحبل السري، والذي يقسم إلى أربع مجموعات. حيث التعرض للإجهاد التأكسدي يتم بواسطة عن $CuSO_4$ والإنتان بواسطة *Staphylococcus aureus*. بعد ساعة واحدة من الحضانة، قمنا بعزل الخلايا البطانية ودرسناها.

النتائج: توضح دراستنا العلاقة بين تعفن الدم وميرنا 23ب، حيث أظهرنا أن مستويات ميرنا 23 ب زادت في الخدج والمواليد الناضجين الذين يعانون من الانتان الدم المبكر ($P < 0.001$ و$P < 0.005$ على التوالي)، ولكنها انخفضت في الإنتان المتأخر

($P < 0.005$). وثبت الارتباط السلبي بين حديثي الولادة الذين ماتوا بسبب الإنتان ومستوى ميرنا 23 ب.

في نموذج خارج الجسم الحي، أظهرنا أن الخلايا البطانية تتصرف بشكل مختلف ضد البكتيريا في جميع الظروف الأربعة. مع البيتاميثازون وبدون CuSO4، لاحظنا انتقال البكتيريا إلى الدم مع انخفاض مستوى miR-23b. يؤدي حقن CuSO4 في الدم إلى حدوث تغيير في نشاط ECs ومحافظة على مستوى miR-23b، والذي يساهم بدوره في الدفاع ضد انتقال البكتيريا إلى الدم.

الاستنتاجات: الانخفاض في مستويات ميرنا 23 ب بلا شك عامل مهم في تطور الإنتان الوليدي. بالإضافة إلى الدور الوقائي للإجهاد التأكسدي الناجم عن النحاس، فالخلايا البطانية المنشطة تعزز الاستجابة المؤيدة للالتهابات في الدم قد تساعد في منع انتقال المكورات العنقودية إلى الدم

الكلمات المفتاحية: الإنتان الوليدي، الخلايا البطانية، أنواع الأكسجين التفاعلية، ميرنا 23-ب.

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Dedications

To My dear parents, for their love and support

Encouraged and supported me in the most difficult times

To those who gave me everything with love Amar and Elkhier

To all my brothers and sisters and their families

To my nephews and nieces

To my dear daughter Ouidjdane

To all my friends

To all my family

To my teachers at different levels

To all newborns affected by sepsis and to all patient

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ABREVIATIONS LIST

$^{1}O_2$: Singlet oxygen

2D: Two Dimension

ADP: Adenosine DiPhosphate

AgNO3: Silver nitrate

AJ: Adherent junctions

ATP: Adenosine TriPhosphate

BCR: B-cell receptor

BPS: phosphate buffered saline

cGMP: Cyclic-guanosine monophosphate

CMV: Cytomegalovirus

$CuSO_4$: Copper sulphate

CuZnSOD: Copper-zinc superoxide dismutase

DNA: Deoxyribonucleic acid

EBV: Epstein-Barr virus

EC: Endothelial cell

FoxO: Forkhead box O protein

GBS: Group B Strep

H_2O_2: Hydrogen peroxide

HOONO: Peroxynitrous acid

HSC: Hematopoietic stem cell

HSV: Herpes simplex virus

HUVEC: human umbilical vein endothelial cells

ICAM-1: Intercellular adhesion molecule-1

IFN: Interferon

Ig: Immunoglobulins

IL: Interleukins

LPS: Lipopolysaccharides

MAPK: mitogen-activated protein kinase

MCP: Chemotactic monocyte protein

MnSOD: Manganese superoxide dismutase

NF-kB: Nuclear factor-kappa B

$NO^{\bullet}$: Nitrogen monoxide

NS: Neonatal sepsis

$O2^{\bullet -}$: Superoxide anion

$OH^{\bullet}$: Hydroxyl radical

PBMC: peripheral mononuclear blood cells

PGI2: Prostacyclin

PMN: Polymorphonuclear leukocytes

ROS: Reactive oxygen sepsis

RSV Respiratory syncytial virus

SOD: Superoxide dismutase

TGF-β: transforming growth factor-β

TNF: Tumor necrosis factor

Treg: Regulatory T

VCAM-1: Vascular Cell Adhesion Molecule-1

VE: Vascular endotheluim

VLBW: Very-low birth weight of infants

VWF: Von Willebrand factor

VZV: Varicella-zoster virus

Introduction

Introduction

Understanding the distribution of causes of death in newborns is important for the identification of appropriate interventions and program priorities. Where augmented in early life (0-6 days of age) with a different causes [1]. The main cause for this is the neonatal immune system. It is characterized by their immaturity, based primarily on innate immunity responses [2, 3].

Each sepsis that occurs in the first 28 days of life is called '*neonatal sepsis*'. Its takes an important place in the public health problem to end the high level of mortality and morbidity [4, 5]. As bacteria and viruses are the most common causative agents; while than the diagnosis of fungi and parasites in sepsis is small compared to bacteria, but important in the etiology of neonatal sepsis [6, 7]. The diagnosis of neonatal sepsis has so far been considered a challenge, it is commonly proven by haemoculture results, which can take many days and suffer from contamination and false negatives [8, 9]. Empirical antibiotic therapy is common, even the consumption of antibiotics by a newborn can increased the risk of the emergence of multi-resistant strains; while delaying or stopping the use of antibiotics may lead to death in the newborn [10, 11].

When we try to understand sepsis in newborns, we must have a good understanding of the mechanism of sepsis in general. Sepsis as a term has undergone many developments in definition over time based on various discoveries.

In 2010, different groups redefined sepsis as acute endothelial dysfunction has been highlighted in response to intravascular and extravascular infection causing reversible or irreversible injury responsible for the failure of many organs [12, 13]. These recent definitions highlight the major role of endothelial cells. these cells play several physiological functions such as controlling vascular tone and blood flow by regulating the local balance between vasodilators, maintaining the fluidity of the blood to prevent thrombosis, controlling the exchange of fluids and macromolecules between blood and tissues, and maintaining the local balance between pro-inflammatory and anti-inflammatory mediators [14]. Endothelial dysfunction is dependent to the decrease of vascular nitric oxide (NO) bioavailability caused by reactive oxygen species (ROS) consumption [15, 16].

The development of science and the methods used in scientific research, led to discoveries of epigenetic modifications important in determining the activity of genes. The main actor of epigenetic regulation are DNA-methylation, histone modification, and non-coding-RNA.

microRNAs (miRNA, miR), are class of small non-coding regulatory RNAs with 19 to 22 nucleotides of length. This small miRNA is a high conserved *single-stranded* RNA, expressed in ubiquitous manner in eukaryotic organisms. Thus, miRNA can inhibit the expression of target genes by their binding to specific mRNA molecules to inhibit their translation to proteins or to degrade them [17, 18].

Usually, to establish a sepsis diagnosis in newborns, a haemoculture is required, as this is the most common traditional criterion. Studies such as the demonstration of the role of ROS or miRNAs in neonatal sepsis remain few.

Indeed, most of them require high-precision technology with very expensive funding in hospitals. In addition, sampling for these studies is a major constraint. For this reason, these studies remain limited.

Based on this, to carry out our work we made the following proposal: In two stages. In the first stage, we performed statistics at the Neonatology Department of Mother & Child Specialized Hospital Establishment (EHS) of Tlemcen to characterize the target group of our study, identify the microorganisms responsible for sepsis, in addition to evaluating the role of microRNA-23b in blood culture instead of obtaining blood directly. In a second step, we relied on an Ex vivo model to study the role of reactive oxygen species in neonates through the creation of oxidative stress by $CuSO_4$ and causes sepsis by staphylococcus aureus in the umbilical cord. In addition to the effect of these molecules on the activity of endothelial cells by 2D (2-dimension) culture, we also measured the rate of miRNA-23b secretion by these cells.

Chapter1: Bibliographic Review

Chapter 1: Literature part

1. Definition

1.1. Sepsis

The first definition of sepsis appear in 1914 by *Hugo Schottmüller* : "sepsis is a state caused by microbial invasion from a local infectious source into the bloodstream which leads to signs of systemic illness in remote organs" [19]; a remarkable note on this definition is that it is based on the power of pathogen. After a while, William Osler was the first who highlighted the central role of the host-response in sepsis: "except on few occasions, the patient appears to die from the body's response to infection rather than from the infection." This observation represents a turning point in modern infection science as it clarifies the role of the host's response to infection [19]. In 2010, different groups redefined sepsis as: "severe endothelial dysfunction syndrome in response to intravascular and extravascular infections causing reversible or irreversible injury to the microcirculation responsible for multiple organ failure" [12, 13]. Judging from these definitions can be argued, an ideal treatment for sepsis should include anti-microbial, anti-inflammatories, and microcirculation protection to avoid the systemic inflammatory response which results in the so-called "*cytokine storm*" which is particularly deleterious in neonatal sepsis [2].

1.2. Neonatal sepsis

Neonatal sepsis (NS) is diagnosed in newborns as young as 28 days old and consist of clinical syndrom that may include systemic signs of infection, circulatory shock, and multiple organ failure [20, 21].

Sepsis is diagnosed most often by haemoculture "*gold standard*", but it is not a mandatory requirement for sepsis. in newborns suspects sepsis with negative blood culture and other sterile site cultures, may be considered to have “clinical sepsis” [9, 22, 23]. According to time of sepsis, neonatal sepsis could be divided into two types, early and late onset sepsis.

1.2.1. Early onset sepsis (EOS)

Sepsis that occurs during the first 72 hours to the first week of life [6, 7, 24, 25]. *Benirschke* had identified this kind of sepsis for the first time in 1959, under the name of “*amniotic infection syndrome*”. In where it was indicated that there is a vertical transition of bacteria from mother to infant before or during delivery [26]. The major risk factor is the premature rupture of the membrane [27] in addition to the *very-low* birth weight of infants (VLBW < 1500 g) [28]. While that intrapartum antibiotic prophylaxis can decreased the risk of EOS [27–29]. The most dominant micro-organisms in the case of EOS: Group B streptococci, *Escherichia coli, Streptococcus viridans, Staphylococcus aureus, Pseudomonas aeruginosa,* Enterococci, and other gram-negative bacilli [29]. The treatment of confirmed patients was an average of 12.3 days with increased administration of cefotaxime, while unconfirmed EOS lasted an average of 6.1 days with gentamicin and ampicillin [9].

1.2.2. Late onset sepsis (LOS)

The LOS occur between 73 h and 28 days after birth [6, 7, 24] and includes all infection postnatal nosocomial or community environment. Its appears after 3 days, with the peak incidence reported to be between the 10th and 22nd day of life [30].

Premature newborns, particularly VLBW preterm infants the most vulnerable group to the disease. This is due to immaturity of the immune system, prolonged hospitalization, and mechanical ventilation for a long time, use of indwelling catheters, endotracheal tubes, and other invasive procedures. The majority of microorganisms isolated during LOS are : *Coagulase-negative Staphylococci, Staphylococcus aureus, Candida albicans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* Enterococci, Group B streptococci [29, 30]. The treatment of confirmed patients lasted for an average of 11.4 days and treatment of unconfirmed LOS lasted an average of 7.8 days. The primarily treatment based to vancomycin and gentamicin, whereas the use of cefotaxime is limited to LOS in some unconfirmed cases [9].

1.2.3. Symptoms of neonatal sepsis

There are no specific symptoms to identify neonatal sepsis. En general including respiratory distress, cyanosis and apnea, fever or hypothermia, hypotonia, feeding difficulties, seizures, lethargy or irritability, bulging fontanel, poor perfusion, bleeding problems, abdominal distention, gauiac-positive stools, hepatomegaly, unexplained jaundice, or more importantly, "just not looking right". Infants with hypoxia–acidosis may gasp in utero and lead to pneumonia and meconium aspiration [29]. More detailed in the **Table .1.1**.

Table .1. 1 Neonatal characteristic of sepsis: bacterial/fungal/viral [31].

LEVEL 1	LEVEL 2	LEVEL 3
Recognized pathogen identified using a validated method and from a normally	Not meeting Level 1 of evidence	Not meeting Level 1 or 2 of evidence

sterile site if an organism normally considered non-pathogenic is isolated from blood cultures : Level 1, requires its identification from at least 2 blood cultures taken from two different sites, or at 2 different times, PLUS 1 of the criteria as per level 2 of evidence	**AND** 3 or more criteria: • Temperature ≥37.5°C or <35.5°C[f] • Tachycardia or new or more frequent episodes of bradycardia • New or more frequent episodes of apnea or increased oxygen requirement or increased requirement for ventilatory support • Lethargy or moving only when stimulated or hypotonia or irritability • Difficulty in feeding or abdominal distention • Pallor or poor perfusion or hypotension • Abnormal White Cell Count or I/T ratio >0.2 • Abnormal platelet count d • Increased inflammatory markers (CRP, procalcitonin)	**AND** 2 or more of the following criteria: • Temperature ≥37.5°C or <35.5°C[f] • Tachypnea or severe chest indrawing or grunting or cyanosis • Change in level of activity • History of feeding difficulty • History of convulsions

	• Metabolic acidosis as defined by a base excess	

1.2.4. Epidemiology

Worldwide, a systematic review that looked at the global burden of neonatal sepsis from 1979 to 2016 showed an annual incidence of 3 million newborn sepsis cases with a mortality rate of 19 % [21]. The World Health Organization (WHO) estimates that more than 1 million neonatal deaths worldwide annually are caused by severe infections, and ~1 million deaths are due to neonatal sepsis or pneumonia alone. Where the Morbidity of NS differs significantly from country to country. In developed countries, The incidence of NS varies from 1 to 5 cases per 1,000 live births, but increases in developing countries, varying from 49 to 170 per 1,000 [32]. Although, approximately 7 % to 13 % of all neonates are worked up for sepsis, but only 3 % to 8 % develop positive cultures [25]. In a study in the United States, approximately 400.000 babies were evaluated and the rate of EOS was found to be 0.98 per 1000 live births, mothers of 57 % of babies diagnosed with EOS were reported to have *group B streptococcal* prophylaxis [33]. In the polyvalent neonatology department wish is located in western Algeria (Tlemcen), the predominant cases were sepsis which ranked (76 %) of all cases. In other study published in 2015; carried out in the department of neonatlogy of Tlemcen; declared that: The majority of infected newborns (91.5 %) received antibiotic therapy on entry, while 22 % of them for suspected EOS [34].

1.2.5 Risk Factors of neonatal sepsis

In addition to the aforementioned reasons, we also find low levels of trans-placental maternal IgG levels in preterm, fetal distress, low APGAR score, resuscitation of the baby, and multiple pregnancies increase the risk of EOS in developing countries. Also, include inadequate antenatal care, high rate of home birth, unsanitary birth and umbilical cord care practices, and late recognition of conditions that pose a risk of infection in the mother or baby. Whereas, many invasive procedures in LOS are present such as: frequent blood sampling, intubation, mechanical ventilation, catheter/probe insertion, insufficient breastfeeding, long-term parenteral nutrition, low stomach acid, and surgical interventions [33]. There are also other common factors associated with pain, among them: chorioamnionitis, early rupture of membranes for more than 18 hours, maternal fever during childbirth (> 38° C), childbirth before 37 weeks of pregnancy, colonization of streptococcus from the maternal group GBS (Group B Strep), and other cases of it augmented the risk of developing GBS infection in the newborn [35, 36].

1.3. Neonatal immune system

The neonatal immune system is a complex structure, evolved in a convoluted step-wise manner [37] **(Figure.1.1)**. In many ways, the immune system early life of newborns is the result of the immune environment during pregnancy in the early stages of embryonic life [38]. Many studies have shown that several perturbations of the immune system development in neonates may be induced by the mother's nutritional imbalance, both deficiency, and excess. Thus, can have a susceptibility to infections at early birth or later-life risk of immune-mediated or inflammatory diseases [39].

The neonatal immune system must first distinguish between self and non-self, and then quickly distinguish between benign or even “useful” non-self like the commensal microbiome and potential pathogens [40].

During this period of immune education, the developing immune system must strike a delicate and pathogen-specific balance between inducing potentially damaging pro-inflammatory responses mediated by type 1 T helper (Th) cells (Th1) and some Th17 type cells. Less damaging inflammatory responses (*e.g,* Th2-like) or even suppressive responses mediated by regulatory T cells (Treg) [40] **(Figure 1.1)**. Induction of suppressive responses may seem counterintuitive when dealing with potential pathogens, such responses appear essential to prevent the fetus from overreacting to maternal antigens or colonization of the neonatal gut and other body surfaces by the normal microbial [40].

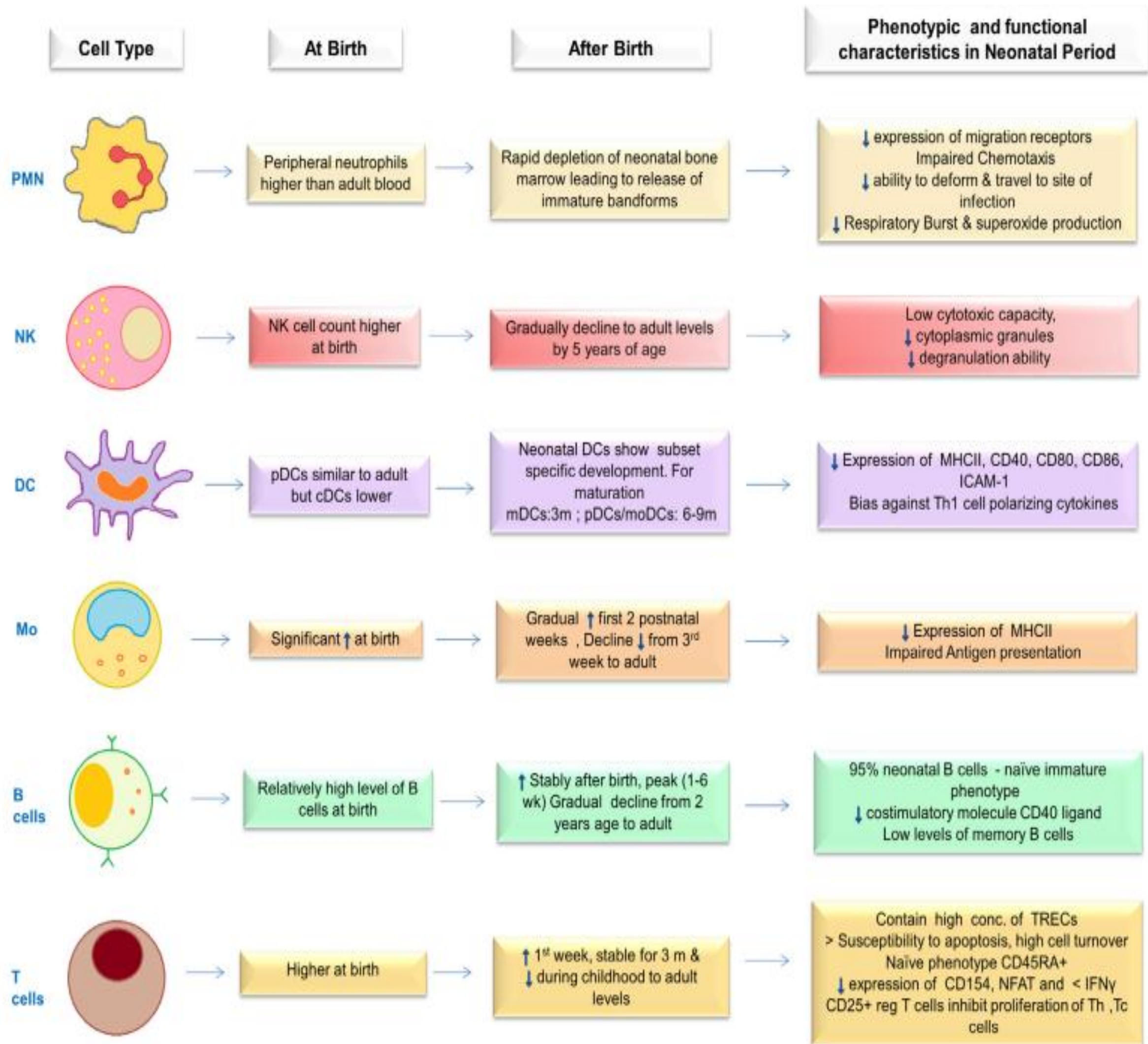

Figure.1. 1 Characteristics of immune cells during the neonatal period. NK: Natural killer, PMN: Poly-morpho-nuclear leukocytes, DC: Dendritic cells, ICAM-1: Intercellular adhesion molecule 1, TRECs: T cell receptor excision circles, NFAT: Nuclear factor of activated T-cells, MHC: Major histocompatibility complex [2].

1.3.1. Adaptive neonatal immunity

Overall, Newborns are developing the immune system with little immunological memory, which increases their vulnerability to infectious agents. Recent advances in understanding of neonatal immunity indicate that both innate and adaptive responses are dependent on precursor frequency of lymphocytes, antigenic dose and mode of exposure [41] **(Figure.1.2)**.

Many studies in different model neonatal, mouse models or human umbilical cord blood cells demonstrate the difference between neonatal and adults immune cells to produce immune responses [41] . Precisely, the adaptive immune response in neonates differs dramatically from that of children and adults **(Figure.1.2)**. Neonatal T cells have been categorized as being both anti-inflammatory and tolerogenic **(Figure.1.2)**, a functional phenotype that appears to be programed into the hematopoietic stem cell (HSC) development of neonates. Moreover neonates possess HSCs whose T cell lineage is biased toward tolerance [42]. T-dependent humoral responses to vaccines and natural infections have been reported to be diminished, delayed, and short-lived in newborns compared to responses in adults [43]. Neonatal T helper demonstrate an excess of Th2 responses (IL-4, IL-5, IL-10) important to allergies, with a decrease production of Th1 cytokines (IFN-γ, IL-2, and TNF-α) implicated counter microbes. Suppression of interferon (IFN-γ) secretion by Th1 cells was shown to be due to higher expression and secretion of IL-4 by Th2 [44].

Multi-potent lymphoid progenitors in human cord blood with CD34+CD7+ and CD34+CD10+CD19+ differentiate to become B cells, by high regulation of multiple factors including cytokines, stromal cells, transcription factors and extracellular matrix components. The maturation and differentiation of fetal B-cells involves activation of transcriptional factors in a stepwise manner, the somatic *V, D, J* and H exons recombination of immunoglobulin genes leading to accumulation of immunoglobulins IgD and IgM molecules on B cell surface [41]. The deficiencies in neonatal antibody production due to various features such as B-cell immaturity, poor B-cell repertoire or reduced strength of BCR (B-cell receptor) signaling. Deficient to produce antibodies upon T-independent antigen stimulation [41].

This deficient can be related to higher expression of negative regulators of BCR signaling or cross-linking of antigen to BCR molecules. Alternatively, by the high density of IgM molecules, low expression of complement factors C3d and CD21/CD22. Further, defects in the nuclear signaling pathway such as NF-κB (nuclear factor-kappa B) [41].

1.3.2. Innate neonatal immunity

The neonatal immune system is characterized by its plasticity and high tolerance, these are due to both the fetal development during gestation as well as significant sudden changes in the fetal environment and enormous exposure to the new antigens and intestinal bacteria and their products. This "*quiescent mode*" of the innate immune system is part of a highly regulated process to fulfill all requirements of the multi-layered process of early life, implemented effectively through the cells of the innate immune system [37]. Neonatal neutrophils have both quantitative and qualitative deficiencies. At birth, the number of neutrophils ranges from $1.5–28 \times 10^9$ cells/L blood, compared to steady state levels of 4.4×10^9 /L in adults [41] **(Figure.1.1)**. Besides, in mononuclear cells from the umbilical cord, deficient the production of IL-12 may have a role in inadequate IFN-γ production, which contributes to the unique susceptibility of neonates to GBS infections [45] **(Figure.1.2)**.

B streptococci, *Escherichia coli, Listeria monocytogenes*, herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), respiratory syncytial virus (RSV), varicella-zoster virus (VZV), *Toxoplasma gondii*, and *Candida* species, can cause infections in utero, intrapartum, and postpartum induce fetal and neonatal innate immune responses [46].

Exposure to recombinant human IFN-γ can activate cord blood neutrophils where enhanced chemotaxis and increased concentrations of free intracellular calcium. These data suggest that this developmental defect in phagocytic cell movement may be the result of an intrinsic defect in IFN- γ production resulting in a deficiency of this critical phagocyte-activating lymphokine [47]. The "*Complement system*" is an important component of innate immunity, all major components of the complement cascade, such as C1q, C4, C3, properdin, and factor B are decreased in newborns. Induced complement system under development and does not function in its full capacity, which may lead to higher susceptibility to the infections and other pathological conditions [2].

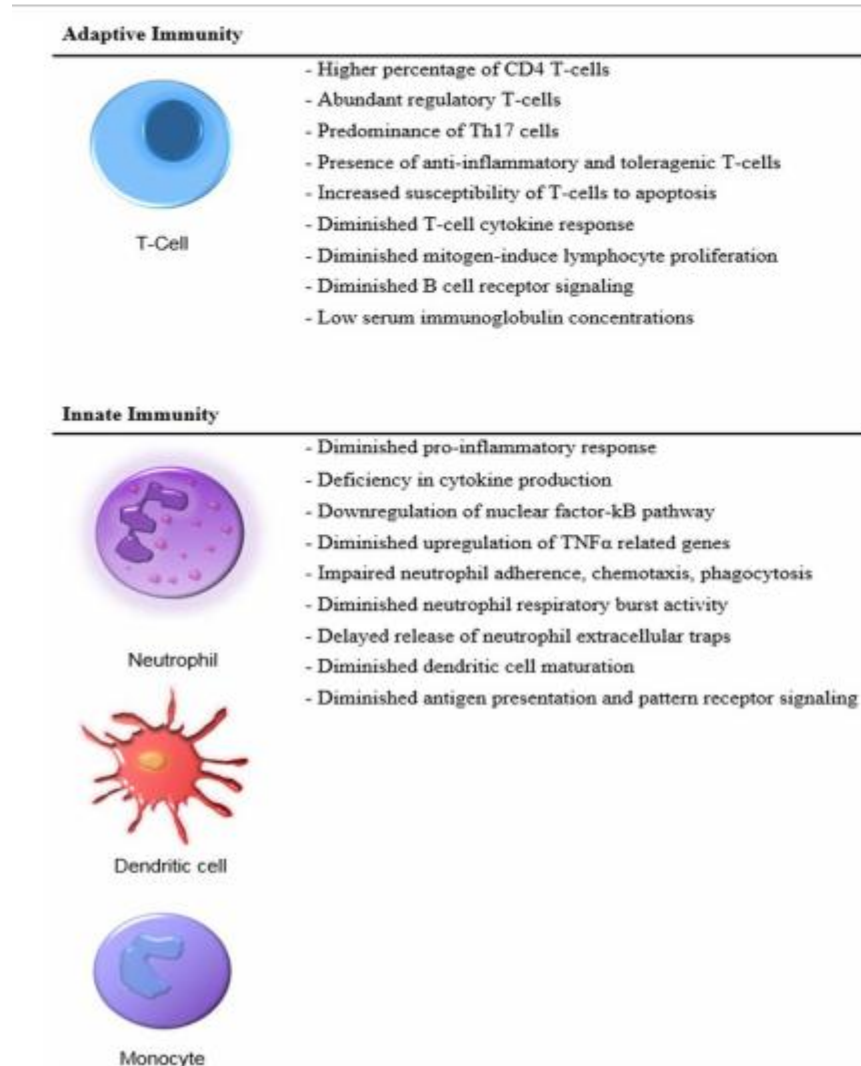

Figure.1. 2 Distinct features of the neonatal immunity [42]. Differences in general characteristics of innate and adaptive immunity in the newborn and main cells implicated

1.4. Endothelial cells

1.4.1. Endothelial cells (ECs)

The endothelium is composed of vascular endothelial cells, which are organized in a single cell layer, on the luminal side of all blood vessels [48]. In previous decades, it has been evident that the endothelium is by no means a passive inner lining of the blood vessels. This "organ"; with a large surface area (~350 m^2) and relatively small total mass (~110 g) [49].

It is actively involved in vital functions such as the control of the degree of vascular relaxation and constriction, the extravasation of solutes, fluids, macromolecules, and hormones, as well as that of platelets and blood cells. Intervenes in the control of vascular tone, where EC regulates regional blood flow. During an immune response, they direct inflammatory cells to foreign materials, areas to be repaired, or to defend against infection [50]. Moreover, the ECs play a major role in inflammation and angiogenesis. Also, to their important role in controlling blood flow, platelet adhesion/aggregation, activation, adhesion, and transmigration of leukocytes. Important morphological, physiological, and phenotypic differences between ECs in different parts of the arterial tree and between arteries and veins, in the various organs optimally support their specified functions in these vascular areas [50].

1.4.2. Structures

The continuous endothelium covers most of the arteries, veins, and capillaries of the brain, skin, lungs, heart, and muscles. ECs are coupled by tight junctions and anchored to a continuous basement membrane. Fenestrated endothelium is associated with a continuous basement membrane and is characterized by the presence of trans-cellular pores 50 to 60 nm wide, which are sealed by a diaphragm 5 to 6 nm thick **(Figure.1.2)**. This is observed in tissues with high trans-endothelial transport or an increased filtration role, such as endocrine and exocrine glands, gastrointestinal tract, choroid plexus, renal glomeruli and renal tubule subpopulations. Discontinuous endothelium is associated with a poorly structured basement membrane and is characterized by the presence of large windows 100 to 200 nm wide without a diaphragm, this occurs mainly in the sinusoidal vascular beds of the liver, but also the spleen and bone marrow [51].

VE-cadherins among cell-to-cell junctions in the vascular endothelium (VE), its surface adhesion glycoproteins that form a "zipper-like" structure at the base of ECs and are connected to cytoskeletal components underlying, such as β-catenin. VE-cadherins composed of adherent junctions (AJ) and tight junctions (TJ) [52]. In addition to VE-cadherins and TJs, other adhesion proteins facilitate the integrity of vascular endothelial barriers, intercellular adhesion molecule-1 (ICAM-1, CD54), ICAM-2, platelet endothelial cell adhesion molecule (PECAM)-1 (CD31), CD34, and endoglin. Facing the vascular lumen, ECs express transmembrane glycoproteins that contribute to the first contact and rolling of leukocytes, mainly P-selectin and E-selectin. Immunoglobulin-like molecules as ICAM-1, ICAM-2 PECAM-1, and VCAM-1, interacting with integrins (expressed on leukocytes) to firm adhesion and extravasation processes [53]. The endothelium in newborns are different in structure and physiology from those in adults and may play an important role in the pathogenesis of neonatal sepsis [54].

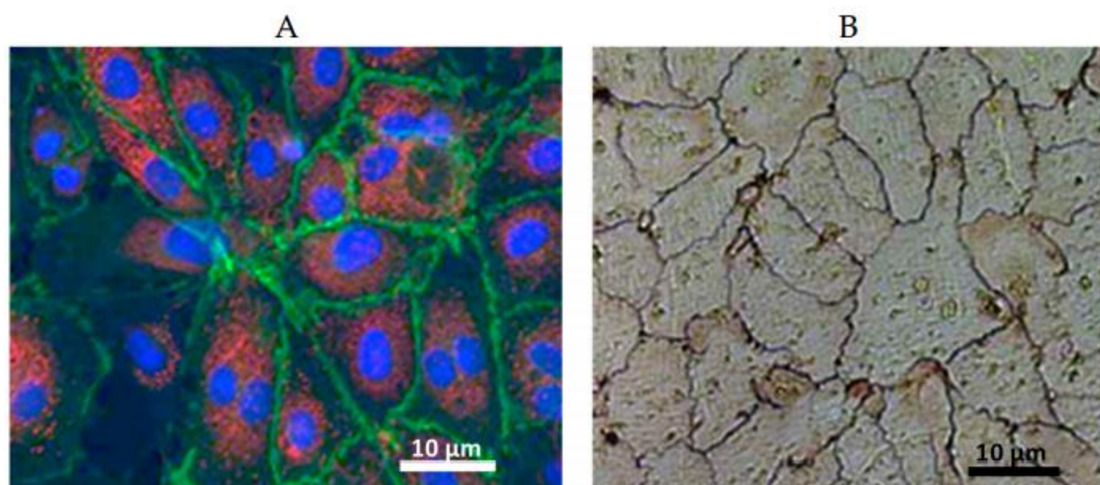

Figure.1. 3 The aspect of endothelial cells under a microscope after cell culture. (A) Immunostaining of an endothelial cell monolayer (cell nuclei in blue, von Willebrand factor in red, vinculin in green); (B) Endothelial cell borders from the confluent endothelial cell monolayer are stained according to Ranvier with silver nitrate (AgNO3) (400-fold primary magnification) [50].

1.4.3. Endothelium and neonatal sepsis

The vascular endothelium in adults represents a key player in the pathophysiology of sepsis and sepsis-associated organ failure, through direct interaction with pathogens, leukocytes, platelets, and the effect of soluble circulating mediators, in part produced by ECs themselves.

Despite abundant evidence that the neonatal immune response to sepsis is distinct from that of adults, comparable knowledge on neonatal vascular endothelium is much more limited. Neonatal endothelial cells express lower amounts of adhesion molecules compared to adult ones and present a reduced capacity to neutralize reactive oxygen species **(Figure.1.3)**. Conversely, available pieces of evidence on biomarkers of endothelial damage in neonates are not as robust as in adult patients, and endothelium-targeted therapeutic opportunities for neonatal sepsis are almost unexplored [54].

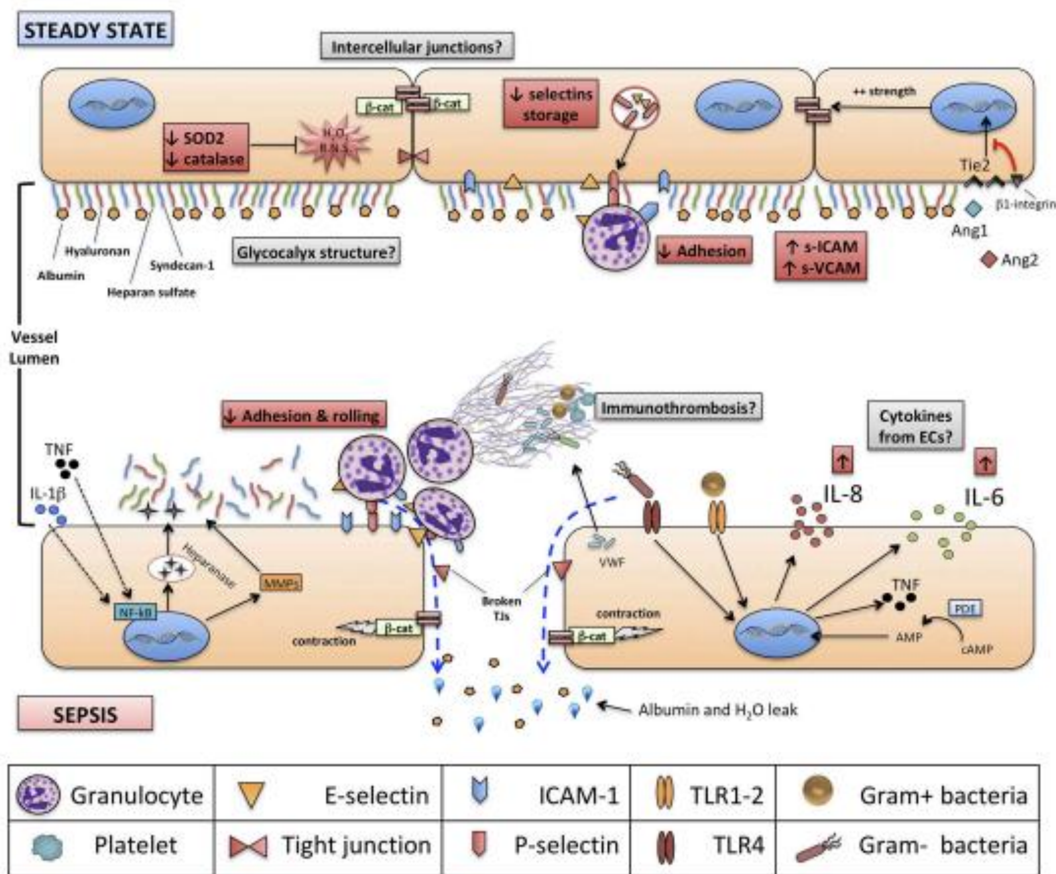

Figure.1. 4 Structure of neonatal VE and interactions with neonatal immune system, at steady state and during sepsis. Red boxes indicate known features of neonatal VE and immune system compared to adults. Gray boxes identify areas for further research. Green boxes identify new experimental endothelium-targeted therapeutic opportunities for neonatal sepsis. NETs, neutrophils extracellular traps; NF-kB, nuclear factor kappa-B; ERK ½, extracellular signal-regulated kinases 1 and 2; β-cat, beta-catenin; SOD-2, superoxide dismutase 2; TRIF, TIR-domain-containing adapter-inducing interferon-β; IL, interleukin; Ang1-2, Angiopoietin 1-2; Tie2, TEK receptor tyrosine kinase; cAMP, cyclic-adenosine monophosphate; AMP, adenosine monophosphate [54]

1.5. Oxidative stress

The first time where the term "*stress*" was used in the biomedical literature to describe the hyperactivity of the hormonal system (corticosteroid of the adrenal cortex). Twenty years later, stress, stress response, and homeostasis as a dynamic equilibrium have gradually developed into a widely used tool for explaining pathophysiologic processes. We now know that in many of these stressful situations, redox processes actually play a major role [55].

Redox imbalance or oxidative stress takes on the same mean. The term "oxidative stress" was created only 30 years ago. The concept is based on earlier work by Selye and inspired by early publications related to oxygen toxicity, often linked to the problem of aging, the metabolism of oxygen (and other) radicals in biological systems, the progressive development of our understanding of mitochondrial physiology, research on "mitochondrial" aging, and the study of redox imbalance in cells and organisms [55].

1.5.1. Definition of oxidative stress

The imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products caused oxidative stress **(Figure.1.5)**. The most practical and operational definition of oxidative stress used until now is given by *Lushchak* “Oxidative stress is a situation when steady state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents” [56]. The commonly ROS are superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$); they are produced as metabolic by-products by biological systems [57].

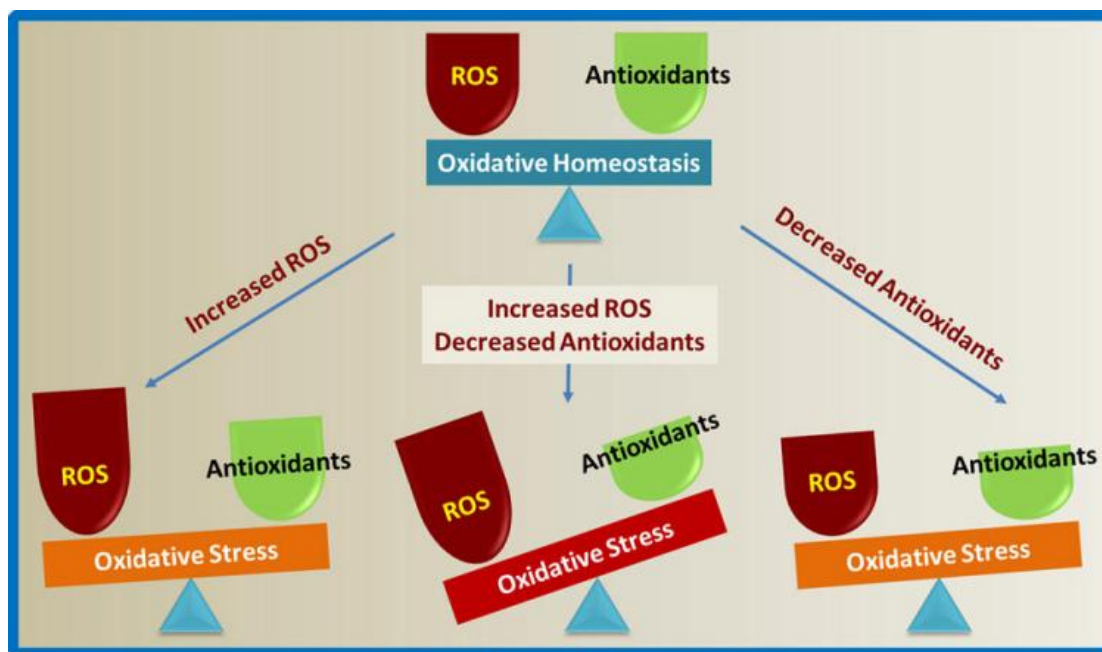

Figure .1.5 Schematic illustration demonstrated the causes of oxidative stress. Either through increased formation of reactive oxygen species (ROS), or through decreased antioxidant defenses, or both [58]

1.5.2. Causes of oxidative stress

Under physiological conditions, the production of ROS is perfectly controlled by the defense systems of our organism: the anti-oxidants / pro-oxidants are in balance. Oxidative stress will result from a situation where the body no longer controls the excessive presence of toxic oxygen radicals. As shown in Table (**Table .1. 2***)*, the sources of oxidative stress can have various endogenous and exogenous origins [59, 60].

Table .1. 3 Endogenous and exogenous sources of oxidative stress.

life style
Smoking Low consumption of fruits and vegetables Alcohol Medicines Contraceptive pill Sun exposure Strenuous or poorly managed exercise
Environment
Pollution Ozone Asbestos Write-offs Contact with carcinogenic substances
Biochemical mechanisms
Xanthine oxidase (ischemia-reperfusion) Inflammation Impaired endothelial function Iron overload Oxidation of hemoglobin Mitochondrial alterations Biosynthesis of prostaglandins Surgeries (Extracorporeal circulation, transplants)

1.5.3. Production of ROS

Free radicals are characterized by highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules. These radicals are produced by all aerobic cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants [60].

There are endogenous and exogenous sources of ROS. Endogenous sources, including the mitochondrial electron and NAD(P)H oxidases. They are also derived from exogenous sources, such as radiation, air pollutants, and some xenobiotics that undergo continuous reduction and oxidation cycles, *i.e.* the redox cycle. ROS levels in a biological system are determined not only by production rates but also by the presence and activities of the cells antioxidant defenses [58] (**Figure.1.6**).

In general, the production of ROS relies on enzymatic and non-enzymatic reactions. Enzymatic reactions able to generate ROS are those involved in the respiratory chain, prostaglandin synthesis, phagocytosis, and cytochrome P450 system [61].

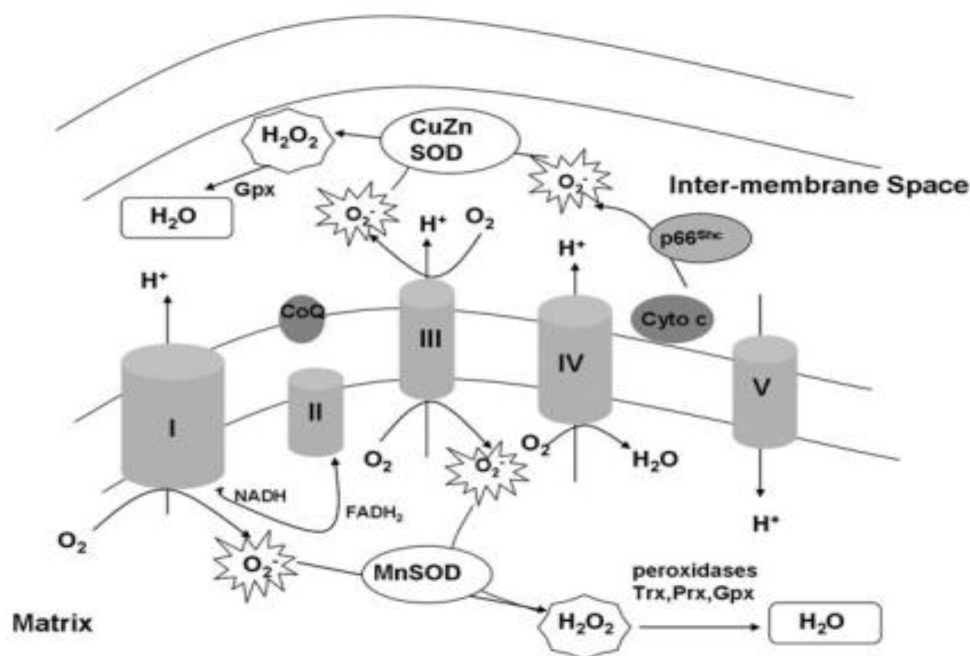

Figure.1. 6 Production of ROS in mitochondria [61] The process of oxidative phosphorylation receives reducing equivalents from the Krebs cycle (NADH to complex I and FADH 2 to complex II) and passes these electrons down the transport chain, ultimately to reduce oxygen to water. The fidelity of the process is incomplete, and the relative fidelity of the process depends on local environmental conditions. As such, oxygen can be reduced to O_2^- in at least three sites within the mitochondria: complexes, I, III, and through p66 Shc. O_2^- that does not escape the mitochondria at this point is rapidly reduced to H_2O_2 by manganese superoxide dismutase (MnSOD) and copper-zinc superoxide dismutase (CuZnSOD) in the matrix and intermembrane space, respectively. H_2O_2 either may leave the mitochondria and react with mitochondria proteins, or may be reduced to H_2O by local peroxidase enzymes. CoQ, coenzyme Q/ubiquinone; cyto C (cytochrome c).

1.5.4. Elimination of ROS

Humans, such as all Mammals, have developed a series of antioxidant defenses to protect vital biomolecules from ROS and related species-mediated damage. In addition, some components derived from dietary sources possess antioxidant activities in biological systems. There are many different kinds of antioxidants in biology and medicine, and they are classified in various ways. For example, superoxide dismutase is an endogenous antioxidant enzyme, whereas vitamin C is a widely known antioxidant derived from a variety of dietary sources [58] (**Figure .1.7**).

There are three major modes of action for antioxidants either by antioxidants that directly scavenge ROS already formed or by antioxidants that inhibit the formation of ROS from their cellular sources; also can by antioxidants that remove or repair the damage or modifications caused by ROS [62] (**Figure .1.7**).

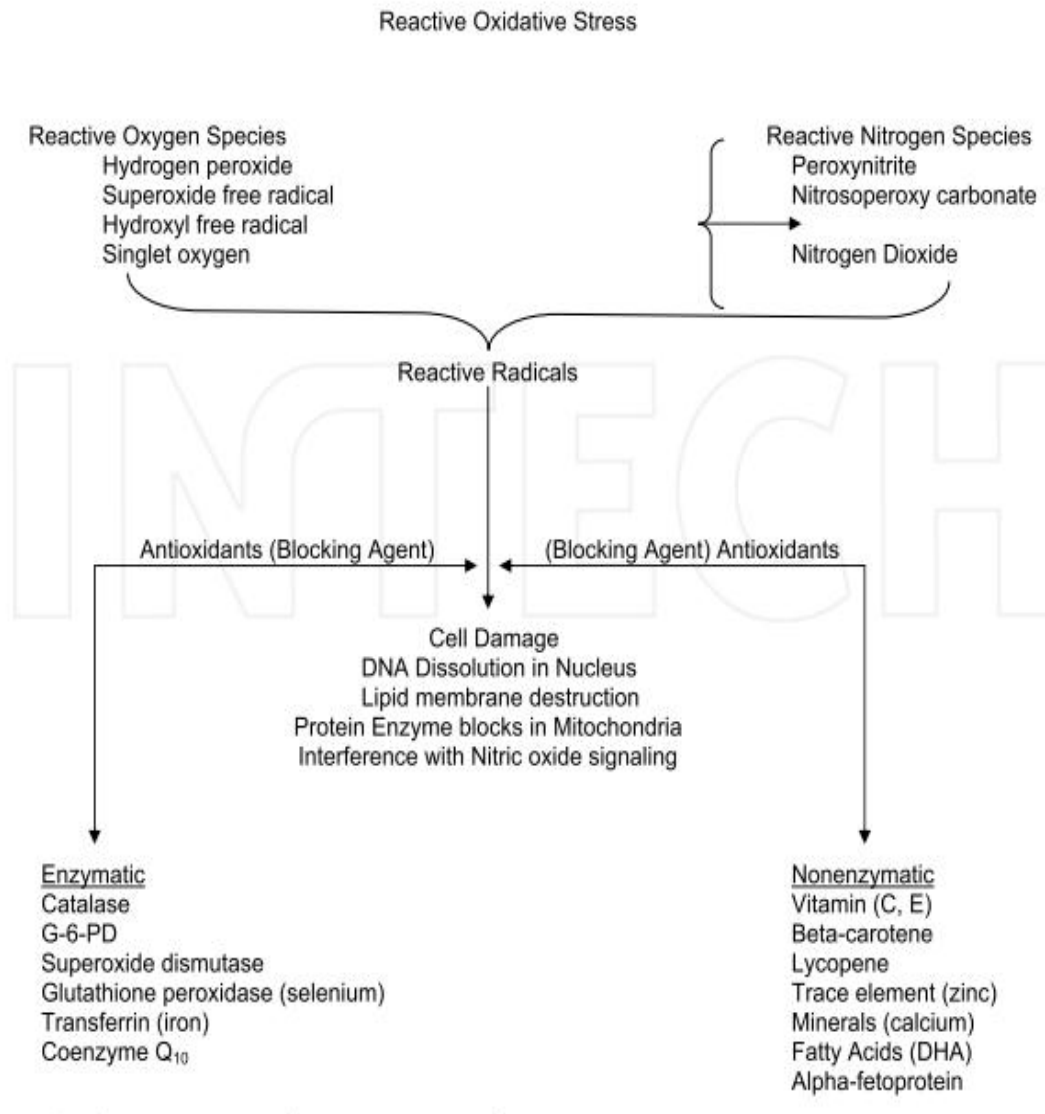

Figure.1. 7 Various Antioxidants Involved in the Elimination of Oxidative Stress [62]

1.5.5. Oxidative stress and sepsis

Sepsis is a complex syndrome characterized by hyper-inflammation, hyper-coagulation, oxidative damage, immune suppression, tissue hypo-perfusion, and hypoxia, as well as multi-organ dysfunction. The pathogenesis of sepsis involves cumulative dysfunction of different immune cells (macrophages, neutrophils, and lymphocytes), endothelial cells, and epithelial cells. Where ROS and reactive nitrogen species (RNS) significantly contribute to the dysfunction of these cells in sepsis [63].

Many studies indicated that ROS can affect the pathogenesis of sepsis by two mechanisms: (a) causing pathologic damage to cells and organs, and (b) modulating the innate immune signaling cascade [64, 65]. Superoxide anion and peroxynitrite play key roles in the pathogenesis of hemodynamic instability and organ dysfunction during sepsis. When any Excessive production of pro-inflammatory mediators, ROS, and proteases by activated neutrophils induced exacerbates sepsis by increasing inflammation, oxidative tissue damage, vascular permeability, and organ injury [66]. In sepsis, an excessive oxidant state leads to reduced ubiquitination and nuclear translocation of the transcription factor, *Nrf-2*, which in turn leads to reduced activation of genes that encode for anti-oxidant enzymes (**Figure 1.8**) [67].

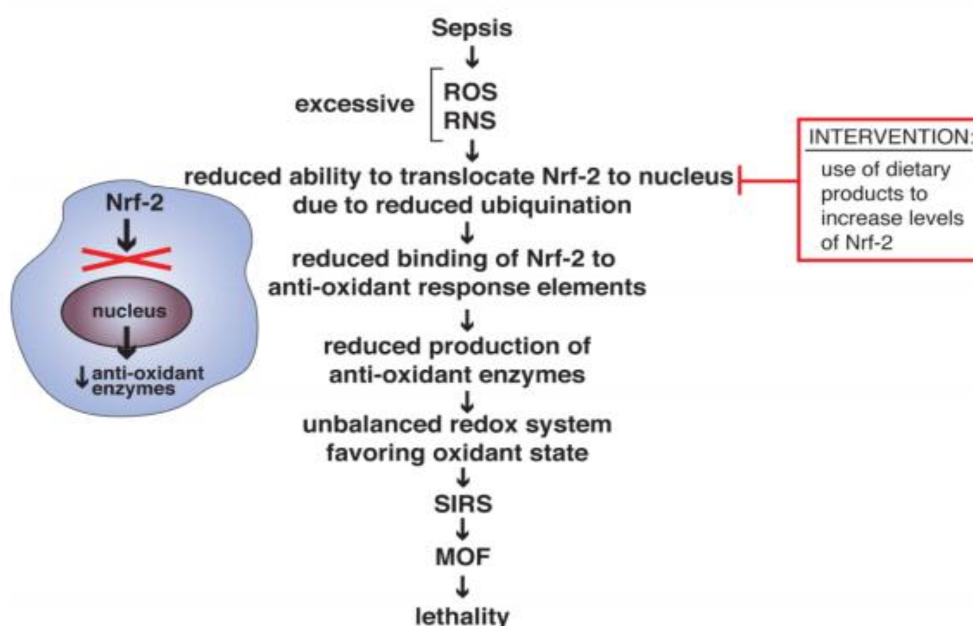

Figure.1. 8 Sepsis-induced defects in redox balance and anti-oxidant enzymes [67]

1.5.6. Oxidative stress and endothelial cells

Inflammatory responses used ROS as mediators, these molecules active cell signaling by increasing the production and release of pro-inflammatory cytokines. Thereby a perpetuation of the inflammatory responses. During an immune response, the inflammatory response is controlled by innate immunity, where, any overstimulation or prolongation of inflammatory responses cause tissue injury and, as such, constitute a major pathophysiological mechanism of a wide variety of human diseases [68].

An imbalance between the generation of reactive ROS and antioxidant defense systems represents the primary cause of endothelial dysfunction, leading to vascular damage in both metabolic and diseases [69].

The first step of alteration is the activation of endothelial, which is characterized by an abnormal pro-inflammatory and pro-thrombotic phenotype of the endothelial cells lining the lumen of blood vessels. This ultimately leads to reduced nitric oxide (NO) bioavailability, impairment of the vascular tone, and other endothelial phenotypic changes collectively termed endothelial dysfunction(s) [69]. Superoxide anion can react with NO inducing to the formation of peroxynitrite $ONOO^{-}$. By $ONOO^{-}$ promotes protein nitration and contributes to the dysfunction and death of endothelial cells [69].

Until now, it is not clear whether increased endothelial cell ROS is a consequence of vascular disease pathogenesis or is sufficient to independently drive disease pathogenesis. If so, endothelial cell ROS production would be a rational target to identify new interventions for the prevention and treatment of structural vascular diseases [70].

1.6. microRNA

1.6.1. Biogenesis of microRNAs

microRNAs, are a class of endogenous non-coding RNAs of about 22 nucleotides that are ubiquitously present in eukaryotic organisms and play an important role in the post-transcriptional regulation of genes [71–73]. These molecules are highly conserved and known by their tissue-specificity expression [74, 75]. The biogenesis of miRNAs is under strict control and their deregulation can lead to various kinds of pathologies. The maturation process of miRNAs in humans involves two consecutive cleavage steps controlled by the enzymes RNaseIII '*DROSHA*' and '*DICER*' [76–78].

The first step takes place within the nucleus, via the enzyme *DROSHA*, with its partner DiGeorge, a critical region of syndrome 8 (DGCR8), to release a hairpin RNA (pre-miRNA) of about 65 nt of length (**Figure 1.9**.). Pre-miRNA is then exported in the cytoplasm and undergoes a second cleavage by *DICER*, releasing a mature miRNA duplex of about 22 nt, within the RNA-induced silencing complex (*RISC*) loading complex. The guide strand, which corresponds to the mature miRNA, is then incorporated into the RISC (**Figure.1.9**). miRNAs and their transcriptional regulators tend to form auto-regulatory loops aimed at controlling their respective levels [76–78]. A stretch of six to eight nucleotides in the 5′ end of the miRNA is determined the specificity of the interaction, its so-called seed region, perfectly complementarity to the target mRNA. The rest of the miRNA sequence is less complementary to the target mRNA, which results in a bulged structure [79]. The miRNA-mRNA interaction is translated by inhibition, degradation, or destabilization is the dominant mechanism for inhibition of protein production while inhibition of translation only modestly represses protein production [80] (**Figure 1.9**).

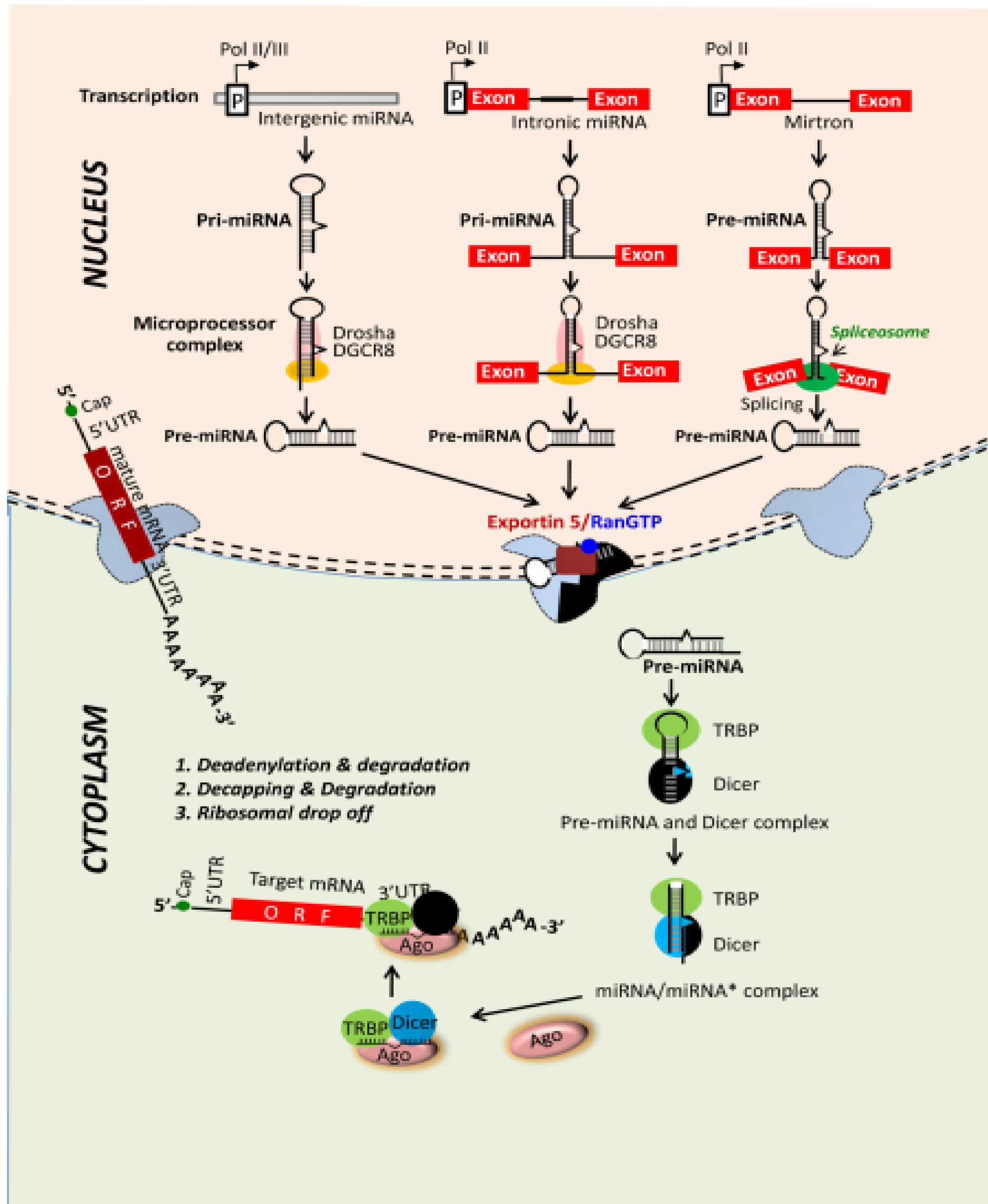

Figure.1. 9 Mechanisms of biogenesis of microRNA [81]

1.6.2. microRNAs and neonatal sepsis

microRNAs have essential roles at different phases of innate immunity, mitochondrial dysfunction, and organ dysfunction in human development, from the beginning of the embryonic phase until aging [82].

In the embryonic phase, as we previously cited, low weight at birth is a risk factor in neonatal sepsis. Moreover, the weight represents an early predictor for metabolic diseases that will determine future complications later in life. nine miRNAs (hsa-let-7a-5p, hsa-miR-374a-5p, hsa-miR-15b-5p, hsa-miR-19b-3p, hsa-miR-23a-3p, hsa-miR-93-5p, hsa-miR-150-5p, hsa-miR-185-5p and hsa-miR-191-5p) upregulated in 40 pregnant women in the first trimester of pregnancy predicted both preterm birth before 37 weeks of gestation and cervical shortening. Moreover, Hsa-miR-150-5p offered the strongest ability to predict preterm birth (AUC=0.8725) and cervical shortening (AUC=0.8514) [83]. In another study, expression of miR-33b, miR-375 and miR-454-3p was different in neonates with normal birth weight, low birth weight, and macrosomia. miR-33b and miR-375 were increased in macrosomia patients and miR-454-3p was overexpressed in both LBW and macrosomia neonates, as compared to normal birth weight babies. These miRNAs are associated target genes: cyclic-guanosine monophosphate (cGMP)-dependent protein kinase (PKG), type 2 diabetes, mitogen-activated protein kinase (MAPK), transforming growth factor-β (TGF-β), and Forkhead box O protein (FoxO) pathways [84].

After transcriptomic data analysis was obtained from peripheral blood samples from infants evaluated for sepsis within 24 h after clinical presentation…, although there were similar major immune pathway deviations in both groups. The analysis revealed the main component of significant differences between patients with early or late sepsis. Time plays an important role in the healthy control and the host's response to sepsis. The time which plays an important role in the uninfected state and the host's response to sepsis [85].

1.6.3. microRNA-23b

The miRNA-23b is involved in various physiological and pathological processes such as autoimmune diseases [86], acute myocardial infarction (AMI), inflammatory heart diseases and sepsis-induced cardiac dysfunction [87, 88], diabetic nephropathy [88], and prostate cancer [89]. In addition, miRNA-23b has been an essential contributor to the activation of cardiac fibrosis to mediate the development of myocardial dysfunction in late sepsis. A recent report suggests that blocking miRNA-23b expression could be an effective approach to prevent sepsis-induced heart dysfunction [90]. miRNA-23b plays an anti-inflammatory role and negatively regulates the inflammatory responses induced by lipopolysaccharide (LPS) by targeting metalloproteinase 10 [91]. The expression of miRNA-23b in the peripheral blood of adult patients with sepsis is related to the manifestation of an inflammatory state and could be used to evaluate the severity and prognosis of the disease [92]. miRNA-23b may play a significant role in the pathogenesis and progression of sepsis by inhibiting the expression of pro-inflammatory factors, including NF- κ B, TNF-α, IL-6, ICAM-1, E-selectin, and VCAM-1 [17].

1.7. Problematic and objectives

1.7.1. Problematic

From the information previously described there is general agreement that neonatal sepsis is a major public health problem in the world in general and in Algeria as well, but a few studies documented NS in Algeria, either as statistics, or as responsible microorganisms and the therapeutic spectrum. Most effective in newborns in the ward.

Despite the research that has been done, neonatal sepsis remains poorly understood because of its complexity and its difference from adult sepsis, either at the molecular or cellular level. miRNA-23b and ROS are co-stimulatory in most physiological and physiopathological processes of endothelial cells. Recent studies have shown that endothelial cells have the main role during sepsis, it can detect changes in their environment and promote changes and adaptations in the structure and function of tissues.

1.7.2. Objectives of this study

- To evaluate neonatal sepsis in Algeria.
- To isolate microorganisms responsible for neonatal sepsis in the department of neonatology of the specialized hospital for children in Tlemcen.
- To determine the efficacy of using haemoculture broth to evaluate microRNA.
- To identify the role of miRNA-23b in neonatal sepsis.
- To develop an umbilical cord model to study neonatal sepsis.
- To test the translocation of bacteria.
- To study the effect of ROS-producing $CuSO_4$ on the role of endothelial cells in our model.
- To measure the different parameters of oxidative stress and the pro-inflammatory cytokines produced by endothelial cells.

1.7.3. Goal

The goal of my project was to build a model to study neonatal sepsis and to decipher the molecular and cellular mechanisms used by endothelial cells during oxidative stress against neonatal sepsis.

Chapter 2: Practical part

Material and methods

Results

Chapter 2

2. Material and methods

To meet our objectives, we followed the method in the diagram below (**figure .2.1**).

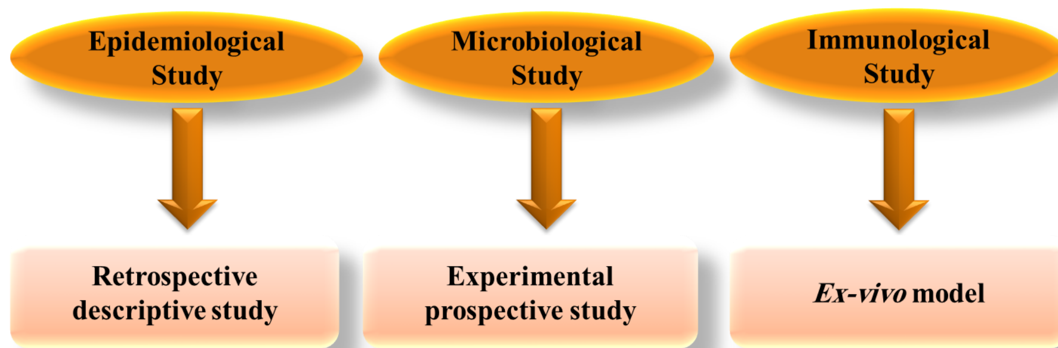

Figure .2. 1 A diagram showing the method of our project

2.1. Epidemiological study

This retrospective study is mainly based on the data registered in our neonatology department of Tlemcen, in a period between 2012 and 2018. Excel 2013 carries out the treatment of the data.

1- Lethality from sepsis = total number of deaths from sepsis / total number of patients from sepsis.
2- Annual incidence of neonatal sepsis = total and specific number of sepsis / number of hospitalizations
3- Mortality in the service = deaths by cause / total deaths

2.2. Microbiology study

2.2.1. Ethical aspects

Our study, with its microbiological and immunological (*ex-vivo* model) aspects, has been approved by the local ethics committee of the University of Tlemcen, Algeria. Parents or legal guardians have given their written informed consent for the samples from all participating infants to be used by the Declaration of Helsinki.

2.2.2. Study population

Fifty-four cases aged up to 28 days with clinical features of sepsis (*e.g.* fever, respiratory distress, bradycardia, tachycardia, convulsions, cyanosis), an association or not with premature rupture of membranes (PROM) [93]. The abnormal amniotic liquid as risk factors in the neonatal sepsis inclusion criteria was recruited in a prospective cohort study. The exclusion criteria included patients without clinical features of sepsis or who received an antibiotic-therapy before sampling. New-borns included 24 females and 30 males. Fifty-four cases were randomly divided into two equal groups of 27 EOS and 27 LOS patients, including nine and six cases of preterm new-borns, respectively.

2.2.3. Samples for haemoculture

Peripheral blood samples (1–2 *mL per patient*) were inoculated into aerobic bottles containing pediatric haemoculture medium (BIOSCAN, Sétif, Algeria) and then incubated at 37ºC for 4–6 h with agitation. Aliquots of 2 mL were collected and stored at – 80°C until RNA extraction **(Figure .2.2)**.

After that, bottles were transferred back at 37° C incubation under aerobic conditions coupled with agitation, for a maximum of 7 days. Subcultures were daily done from the

first to the 7th day. Cultures were reported as negative when they did not yield any growth at the end of 7 days [94] **(Figure.2.2)**.

2.2.4. Isolation of etiologic agents

Blood samples were collected from each patient dispensed into blood culture bottles incubated for a maximum of 7 days. Bottles with growth signs, such as hemolysis, turbidity, clot formation, gas production, were then sub-cultured on blood agar, chocolate, *Chapman*, and *Mac-Conkey* agar plates. The chocolate agar plates were incubated in a candle jar, while the blood agar and *Mac-Conkey* agar plates were incubated aerobically [95]. Significant bacterial isolates were carried out using Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (*MALDI-TOF MS*).

2.2.5. *MALDI-TOF MS* identification

Bacterial species were directly identified from one bacterial colony; each colony was covered with 2 ml of matrix solution without other supplements and extracted as previously described [96]. An isolate was considered correctly identified at the species level by using *MALDITOF MS* (Bruker Daltonics) if two spectra had scores of ≥ 1.9. Uncertainly identified isolates at the species level (scores of < 1.9) were identified with certainty by *MALDI-TOF MS* analysis of two additional spectra. The second run of *MALDI-TOF MS* identification with four spectra was done for unsatisfied species identification in the *MALDI-TOF MS* period.

2.2.6. Evaluation of miRNA-23b

2.2.6.1. Total RNA isolation

Total RNA extraction, including miRNA, was performed with a minimum of 200 µL of cell-free supernatant obtained after centrifuging an aliquot at 1200 rpm for 10 min using the miRNeasy Serum/Plasma kit (Qiagen, Valencia, Spain) according to the manufacturer's protocol. RNA was eluted with 20 µL of RNase-free water and was then quantified in a NanoDrop ND 2000 UV spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.2.6.2. Reverse transcription PCR and Real-time qPCR

Total RNA (1 µL) was converted into complementary DNA (cDNA) by reverse transcriptase using the miRNA TaqMan reverse transcription kit and miRNA-specific stem and loop primers (Part No. 4366597, Applied Biosystems. Inc., CA, USA). Real-time PCR was performed in an Applied BioSystem 7900HT Thermocycler (Applied Biosystems/Thermo Fisher, USA) with 40 cycles. The primers herein used were designed for miRNA-23b, and U6 snRNA was used for standardization purposes by the delta-delta CT method ($2^{-\Delta\Delta CT}$) [97].

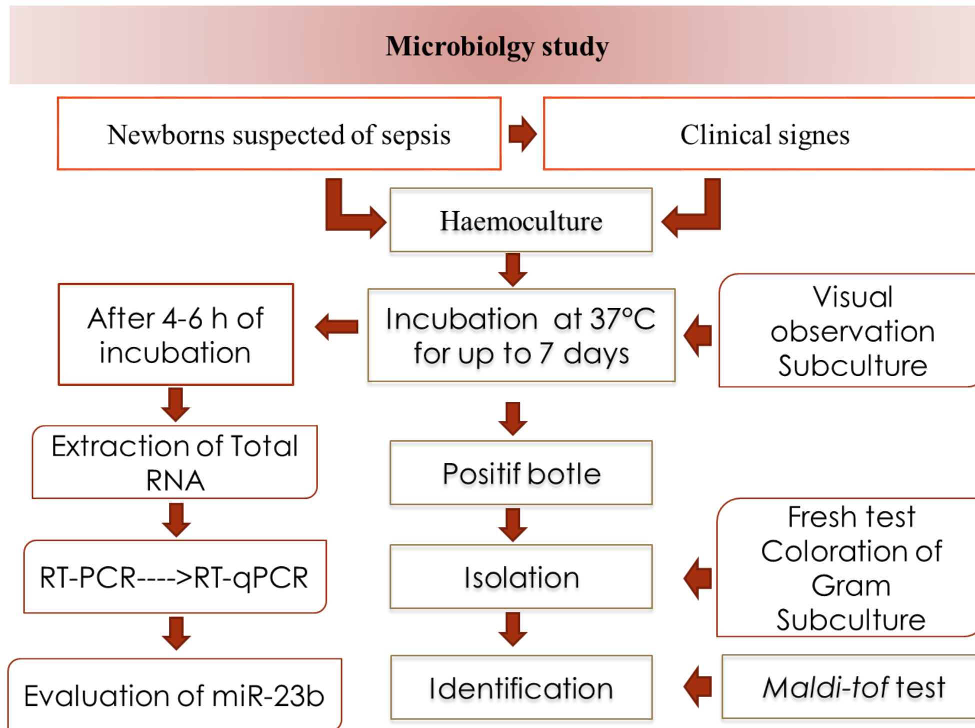

Figure.2. 2 Planning of prospective study in the microbiology part

2.3. Immunological study (*Ex vivo* model)

2.3.1. Bacterial strains

For this experience, we chose *S. aureus* (ATCC # 6538) to provoke sepsis, which was reconstituted following manufacturer instructions in 10 mL sterile Brain Heart Infusion Broth (BHIB). Before each assay, microbial stocks were diluted to a working concentration of 1×10^5 microbes ml^{-1}, 50µL of concentrations 10^5 colony forming units (CFU) mL^{-1} added to 250µL of Tryptic soy broth (TSB) and incubated at 37°C [98].

2.3.2. Human umbilical cord and cord blood sample

The study included umbilical cords from full-term neonates (36 + 6 weeks of gestation) by normal delivery, showing no signs of infection, collected from healthy donor mothers. These umbilical cords were treated with heparin [99], to prevent blood coagulation, and placed in a transport buffer [100].

2.3.3. Development of the model

The human umbilical cord was cut into 4 pieces of identical length to obtain four experimental pieces. The first piece was not treated with $CuSO_4$ and not-infected with *S. aureus* (negative control). While the second piece was untreated with $CuSO_4$ and infected with *S. aureus*. The third piece was treated with $CuSO_4$, and not infected with the bacterium. The fourth piece was treated with $CuSO_4$ and infected with *S. aureus*. For the two pieces infected by *S. aureus* on injecting into the Gelley of Wharton Betamethasone before the injection to bacteria in all of the experiments. $CuSO_4$ is used to generate oxidative stress at a concentration of 10 µM [101], $CuSO_4$ injected into the blood of the umbilical cord vein. Four pieces were incubated in a water bath at a temperature of 37 ° C for 1 hour. Umbilical cord pieces were immersed in phosphate-buffered saline solution (PBS) and incubated for 1 hour at 37C° and 5% CO_2 under agitation [102]. After one hour of incubation, we extracted the venous blood and tested if the bacteria passed through the vessel vein to the blood. infection‘s control was performed on Chapman's medium **(Figure 2.3)**.

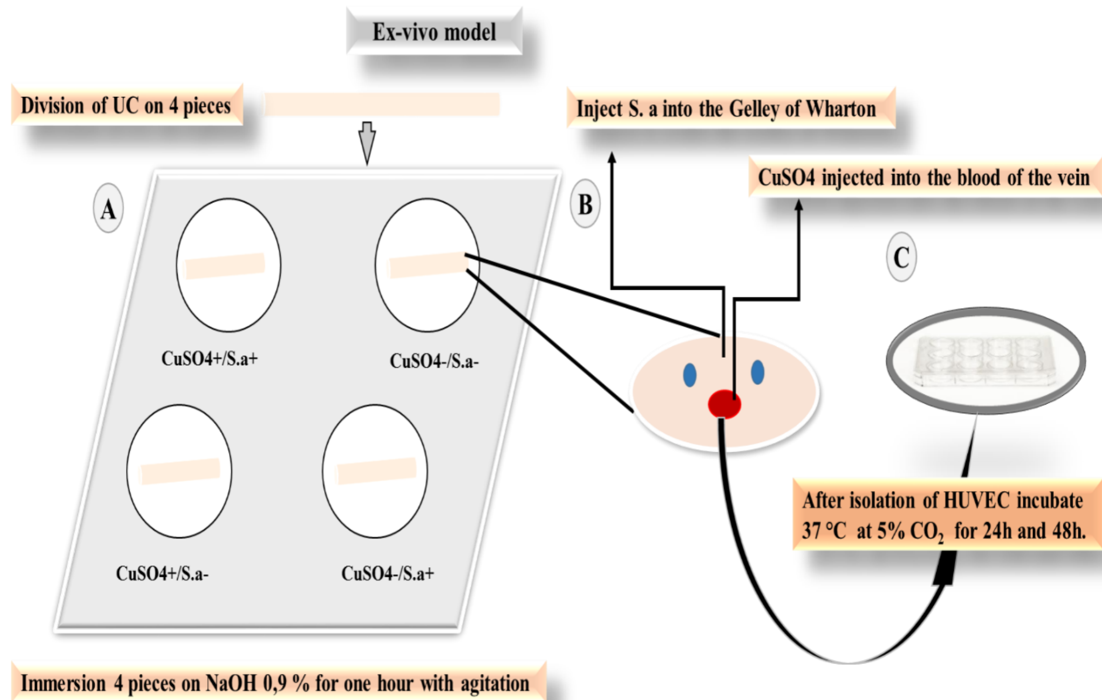

Figure.2. 3 An illustration of *ex-vivo* model. A: First step preparation of pieces and incubation for 1 hour. B: Schematic diagram showing how to inject both of $CuSO_4$ in blood and *S.aureus* in the Wharton's jelly. C: isolation of HUVEC and incubation.

2.3.4. Isolation of human umbilical vein endothelial cells

The Davis *et al*'s protocol (2007) was used to isolate endothelial cells from the umbilical vein [103]. After removing excess blood, the umbilical cord vein was treated with 20 mL of Hanks Balanced Salt Solution (HBSS) (Sigma Aldrich Co., St. Louis, USA), and then incubated with 10 mL of collagenase (GIBCO BRL, Life Technologies, France). Subsequently, the umbilical cord was placed in Dulbecco's Phosphate-Buffered Saline (DPBS), at 37 °C for 15 minutes in a water bath. After incubation, the endothelial cells were collected and placed in supplemented Dulbecco's Modified Eagle's Medium (DMEM), with 10% fetal calf serum (FCS)1 ml of penicillin/ streptomycin [103]. The cells were seeded in 24 well plates (Falcon, corning, USA) at 2×10^6 cells/mL per well at 37°C and 5% CO_2 for 24h and 48h.

2.3.5. Cell viability assay

Cell viability was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT assay kit, Sigma-Aldrich Inc, St Louis, MO, USA).

2.3.6. Cell lysis assay

Cells were treated for 30 min with 500 µL of 0.1% Triton X 100. Reaction stopped by the addition of a mixture containing Tris-HCl and MnCl2 (v/v, 500 µL).

2.3.7. Arginase activity assay

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) activity was measured using a spectrophotometric method based on the determination of the urea levels in cell lysates as described with slight modifications. After incubation at 56 °C for 10 min, 25 µL of activated cell lysates incubated at 37 °C for 1 h with 200µL aliquot of arginine buffer (10 mM L-arginine, pH 6.4). Finally, 750 µL of acetic acid was added to stop the reaction, and the concentration of the urea generated by the arginase was determined at 600 nm using a commercial kit (UREA/BUN-COLOR, BioSystems, S.A. Barcelona, Spain). The enzyme activity is expressed as µmol urea per mg protein per 60 min.

2.3.8. Intracellular free calcium ions assay

The levels of intracellular free calcium ions ($_{if}Ca^{2+}$) were determined on cell lysates. It was determined spectrophotometry at 560 nm against a blank using a commercial kit (BioSystems S.A. Barcelona, Spain). The levels of $_{if}Ca^{2+}$ are expressed as mg/mg proteins.

2.3.9. Catalase activity

The catalase activity was determined according to the method of Aebi (1974) [104]. In this method, the measurement of the decomposition rate of hydrogen peroxide by spectrophotometer and the absorbance was monitored at 420 nm for 3 minutes.

2.3.10. Nitric oxide (NO) assay and endothelial nitric oxide synthesis (eNOs)

The NO production levels were measured on supernatants constructed on the sensitive colorimetric Griess reaction measuring the accumulation of oxidative metabolites (NOx, nitrite, and nitrate). The absorbance was read at 540 nm with an ELISA plate reader (Biochrom Anthos 2020, Cambridge, UK). The concentration of nitrite was determined using a standard curve constructed with sodium nitrite solution ($NaNO_2$) [105]. eNOS activity were calculated from NO concentration per mg of proteins per 30 min.

2.3.11. Adhesions molecules analysis

The intercellular soluble adhesion molecule 1 (sICAM1) and CD62E (selectin) were tested in the supernatant by ELISA, using a commercial kit, according to the manufacture's instruction (Sigma Aldrich Co., St. Louis, USA) and Human E-Selectin ELISA Kit (Sigma Aldrich Co., St. Louis, USA). Optical densities measured at 450 nm with an ELISA plate reader (Biochrom Anthos 2020, Cambridge, UK). The limits of detection were 150 pg/mL and 30pg/mL respectively.

2.3.12. Evaluation of miRNA-23b

We followed the same method that we did in the first part of microbiology. With the use of the supernatant of cell culture, in 24 and 48 h of incubation.

2.3.13. Histology study

After the experiment, we collected samples for histological evaluation. Section of umbilical cords was taken before and after enzyme treatment with collagenase and fixed in 10% buffered formalin. The pieces of the umbilical cord (histological material) are fixed, included, cut, and stained (hematoxylin-eosin, H&E), to be able to observe it under a microscope optic (B-150 OPTIKA). The stained were assessed using an inverted cell imaging fluorescence microscopy station (Floid Cell Imaging Station, Thermo Fischer Scientific, MA USA).

2.3.14. Statistical analysis

In the microbiological part (evaluation of miRNA-23), the results represented the mean (± standard error) of the median values of three independent replicate experiments. Analyses of variance were carried out by Mann-Whitney *U* or Kruskal–Wallis non-parametric tests. In the *ex-vivo* model, all data were expressed as the means ± standard error of the mean (SEM). The hypothesis of data distribution was examined by non-parametric tests, Mann Whitney *U* or Kruskal–Wallis [106]. *P*-value < 0.05 was considered statistically significant. I used GraphPad Prism 8.0.1 (244).

Results

2.4. Results of epidemiology study

From general observations, there is a large percentage of children who are hospitalized for several reasons (13,751 children less than 28 days old). 42,97 % where females and 56,99 % males. Between 2013 and 2014, we noticed a decrease in the number of newborns being accepted into the department. Whereas, in the remaining years, an increase in children was recorded. As for the other years, there has been a decrease, due to the establishment of new units: general care intensive care and reanimation, in addition to the increase in the medical staff, and these updates made it possible to increase the rate of admission of patients as well as improve the medical level of the department. We have also noticed a decrease in the number of cases of sepsis, except for the mortality rate, which remains a major barrier in this department. Lethality, annual incidence, and sepsis neonatal mortality are described in the **Table 2.1.**

During this period, the use of haemoculture such as a tool to evaluate sepsis was recorded in 2016, as for subsequent years it was limited to late onset sepsis exactly suspected to nosocomial sepsis. The most common bacteria identified are *Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli,* and *Staphylococcus aureus.* Our study is the first of its kind, focusing on both early and late neonatal sepsis.

Table .2. 1 Epidemiological data 2012-2018.

			Sepsis		Lethality by sepsis				
Years	**Total patients**	**Total death (%)**	**EOS**	**LOS**	**Total (%)**	**0-6 days**	**7-28 days**	**Etality**	**Incidence**
2012	1931	321 (16,62)	304	106	38 (11,83)	21	17	0,093	0,212
2013	1622	281 (17,32)	253	57	24 (8,54)	18	6	0,077	0,191
2014	1654	299 (18,08)	121	24	16 (5,35)	11	5	0,110	0,088
2015	1935	340 (17,57)	113	44	23 (6,76)	17	6	0,146	0,081
2016	1904	311 (16,33)	132	25	22 (7,07)	15	7	0,140	0,082
2017	2302	344 (14,94)	98	48	22 (6,39)	16	6	0,151	0,063
2018	2403	350 (14,56)	103	52	24 (6,86)	19	5	0,155	0,065

2.5. Results of microbiology study

2.5.1. Description of cases

Among 2,561 admitted newborns; during a 12-month period in 2018; in the Neonatology Department of Mother & Child Specialized Hospital Establishment of Tlemcen (northwest Algeria), only 9,91 % (254 new-borns) were recorded with sepsis. Fifty-four haemocultures were used in this study, 27,8 % premature, and 72,2 % at term. Gender, temperature, heart rate, respiratory rate, glycemia, and cesarean *vs* vaginal delivery characteristics did not mark any statistical differences between the control and the positive haemoculture groups. However, the neonates' weight in the two types of sepsis becomes significantly different in contrast to C reactive protein (CRP) which was

significantly different in the early onset sepsis neonates. The clinical information of the 54 included patients is shown in the **Table 2.2**.

Table .2. 2 Characteristics of the new-borns patients with sepsis

	Full-term patients				Premature patients			*p*
EOS/LOS **(n = 27, 27)**	Co/NH (9, 6)	SP/PH (7, 12)	DP/PH (2, 1)	DP/NH (0, 2)	Co/NH (4, 0)	SP/PH (2, 6)	DP/PH (3, 0)	
Gender (M/F)								
EOS	5/4	4/3	1/1	-	1/3	1/1	2/1	NS
LOS	6/0	6/6	0/1	2/0	-	2/4	-	NS
Weight (kg)								
EOS	2,87 ± 0,25	3,16 ± 0,25	3,30 ± 0,1	-	2,01 ± 47,3	1,43 ± 0,16	1,63 ± 0,33	<0,001
LOS	3,17 ± 0,35	3 ± 0,25	2,5 ± 0	2,9 ± 0,2	-	1,58 ± 0,19	-	<0,0001
T (° C)								
EOS	36,17±0.45	35.05±0.58	34.5 ± 0.9	-	36.35±0.85	33.07±1.28	36.17±0.45	NS
LOS	37.33 ± 1.16	38.3 ± 0.44	37.4 ± 0	36.08 ± 1.25	-	35.12 ± 0.74	-	NS
HR (BPM)				-				
EOS	136 ± 5.5	143.6 ± 7.1	125 ± 7.0	-	120.5 ± 14.8	125 ± 5.0	150 ± 5.8	NS
LOS	151.8 ± 11.0	140.3 ± 5.0	180 ± 0	135 ± 5	-	132 ± 5,19	-	NS
RR (BrPM)				-				
EOS	55,3 ± 4,9	55,7 ± 6,5	38 ± 4		58 ± 2,7	42 ± 2	62,67 ± 2,7	NS
LOS	50,7 ± 5,7	52,5 ± 1,8	52 ± 0	42 ± 2	-	47,5 ± 4,56	-	NS
Gly (mg/dL)								
EOS	0,95 ± 0,16	0,63 ± 0,09	0,52 ± 0,42		0,73 ± 0,15	-	0,45 ± 0,08	NS
LOS	1,12 ± 0,09	0,65 ± 0,06	-	0,66 ± 0,12	-	0,57 ± 0,06	-	NS
CRP (mg/dL)								
EOS	25,78 ± 5,96	41,17 ± 12,55	-	-	63 ± 44,12	-	-	<0,0001
LOS	47 ± 36,39	39,87 ± 7,73	-	42 ± 18	-	54,67 ± 15,89	-	NS
VD *vs*, CD				-			-	
EOS	5/4	5/2	1/1	-	2/2	1/1	1/2	NS

LOS	5/1	1/1	1/0	2/0	-	3/3	-	NS

Data are presented as mean±standard error of the mean (X ± SEM). BPM: beats per minute, BrPM: breaths per minute, CF: Cardiac frequency, Co/ NH: control new-borns with negative haemoculture, CRP: C-reactive protein, DP/NH: patients who died with negative haemoculture, DP/PH: patients who died with positive haemoculture, EOS: early onset sepsis, F: female, Gly: glycaemia, HR: Heart rate, EOS: early onset sepsis, LOS: late onset sepsis, M: male, NS: not significant, RR: Respiratory rate, SP/PH: patients who survived with positive haemoculture, VD *vs.* CD: vaginal *vs.* caesarean delivery.

2.5.2. Isolated microorganisms

Among 37 cases, 33 strains were identified, including 19 strains of Gram-positive, 13 strains of Gram-negative bacteria, and 1 strain of fungi, accounting for 57,57 %, 39,39 %, and 3,03 % of cases, respectively. Among the Gram-positive bacteria, *S. epidermidis* accounted for 21,05 % of cases, *S. hominis* accounted for 26,31 %, and *S. aureus* accounted for 15,79 %, whereas other bacteria including *Enterococcus faecalis*, *Bacillus cereus,* and *Bacillus pumilus* occurred at low rates. Additionally, 47,37 % of coagulase-negative Staphylococcus (CONS) were detected. Out of all the Gram-negative bacteria, *Klebsiella pneumoniae* accounted for 46,15 % of cases; also, 15,38 % of *Enterobacter cloacae* were detected, *Stenotrophomonas maltophilia, Cronobacter sakazakii,* and *Pantoea agglomerans* along with 1 strain of fungi *Candida parapsilosis*. The specific composition is present in the **Table 2.3.**

Table .2. 3 Isolated microorganisms in neonatal sepsis

Bacteria	Name	Cases	Percentage %
Gram-positive bacteria	*Staphylococcus epidermidis*	4	12,12
	Staphylococcus hominis	5	15,15
	Enterococcus faecalis	3	9,09
	Staphylococcus aureus	3	9,09
	Bacillus pumilus	2	6,06
	Bacillus cereus	2	6,06
Gram-negative bacteria	*Klebsiella pneumoniae*	6	18,18
	Stenotrophomonas maltophilia	3	9,09
	Enterobacter cloacae	2	6,06
	Pantoea agglomerans	1	3,03
	Cronobacter sakazakii	1	3,03
Fungi	*Candida parapsilosis*	1	3,03

2.5.3. Evaluation of miRNA-23b in haemoculture

Below we will present the results obtained by measuring the level of miRNA-23b, among newborns at term and premature in early and late sepsis.

2.5.3.1. Changes in expression levels of miRNA-23b in early onset sepsis

miRNA-23b expression levels in neonatal sepsis samples. As **figure 2.4** show compared to the control group, miRNA-23b expression levels were significantly different in neonatal sepsis samples, either at term or in premature neonates ($P < 0.001$ KW) **(Figure 2.4)**. The expression of miRNA-23b decreased significantly in neonates who died of sepsis ($p < 0.0001$, $p < 0.05$ at term and premature infants respectively), and significantly increased in survived neonates with positive blood culture ($p < 0.005$, $p < 0.001$), indicating that miRNA-23b may contribute to the pathogenic process of neonatal sepsis.

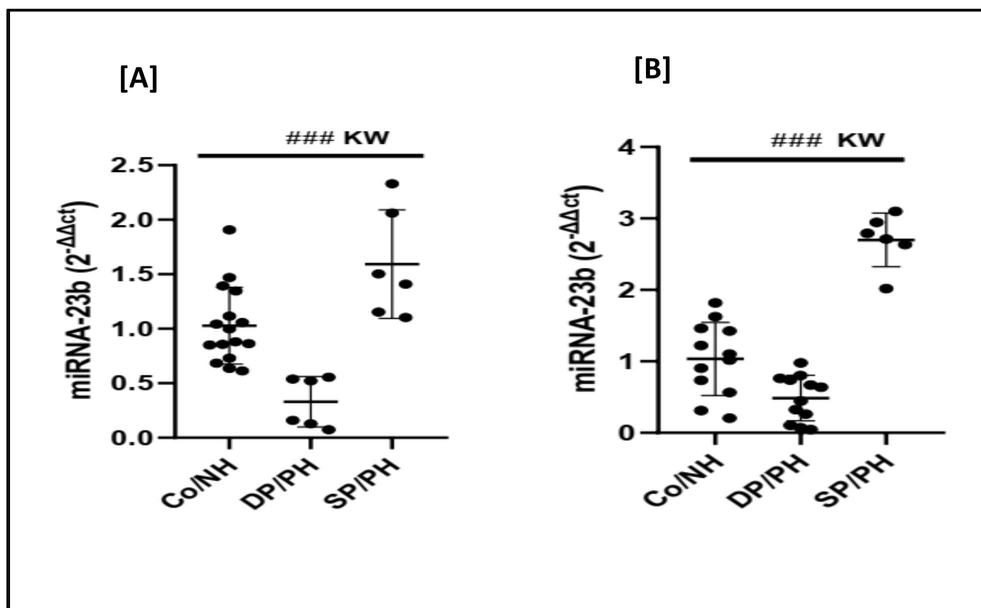

Figure.2. 4 Changes in the miRNA 23b expression levels in early onset sepsis

2.5.3.2. miRNA-23b expression level Changes of in late onset sepsis

As shown in **Figure .2.5**, compared to controls, the expression of miRNA-23b in LOS decreases significantly in both dead and surviving newborns with positive blood culture $p < 0{,}005$, $p < 0{,}05$. Only one single case had the clinical signs of sepsis, where the neonate did not survive but the haemoculture was negative. We recorded in this specific case a significant decrease in the level of miRNA-23b with a negative blood culture ($p < 0{,}05$). We can consider this case as probably haemoculture false negative, because of the time of sampling according to Başustaoğlu *et al* (2019) [107]. Besides, in premature neonates with positive blood culture, the level of miRNA-23b significantly decreased when compared to control neonates with negative haemoculture. *P*-values with Kruskal-Wallis tests were < 0,01.

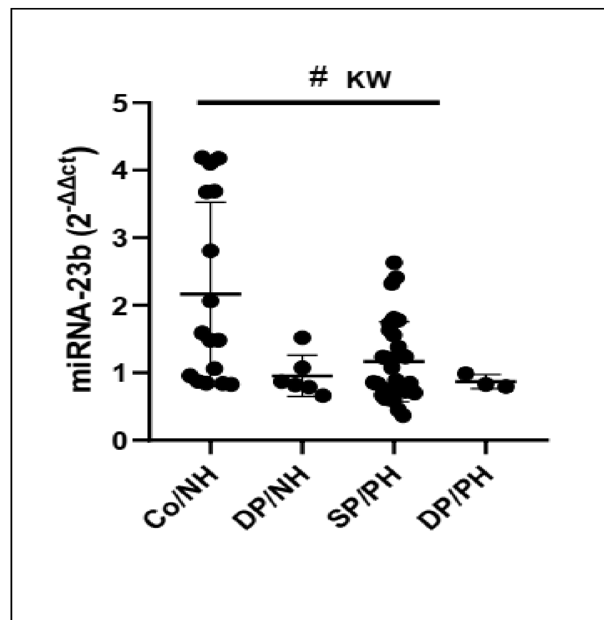

Figure.2. 5 Changes in the miRNA 23b expression levels in late onset sepsis

2.5.3.3. Change in miRNA-23b expression levels in newborns at two different stages

The obtained results (**recorded in Figure .2.6**) show that comparison between early and late sepsis states led us to think back to the starting point before the appearance of sepsis. Figure.2.6 shows the level of miRNA-23b expression at the first 72 h of life and after that time in control patients. A significant increase in the level of miRNA-23b after 72 h of life $p < 0,05$ compared to the first 72 h of life.

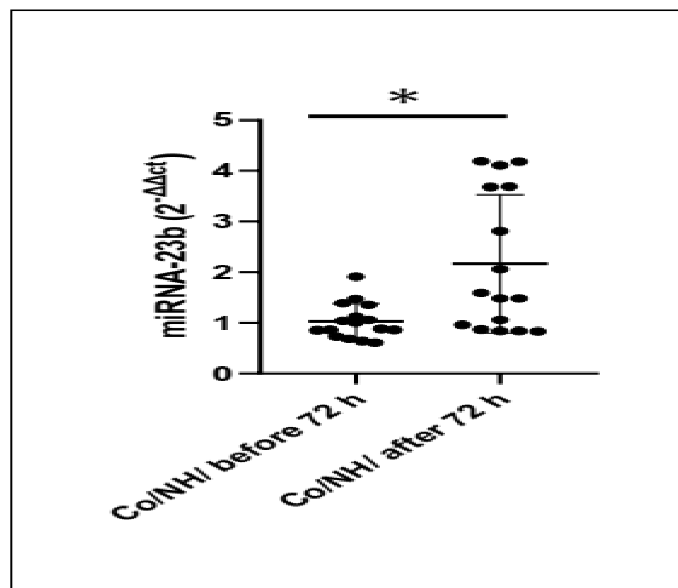

Figure.2. 6 change in the miRNA-23b expression levels in newborns at two different stages

2.5.3.4. Correlation between miRNA-23b expression and dead newborns with sepsis

We show in **Figure.2.7**, the negative correlation between the miRNA-23b and death with early neonatal sepsis in premature and at term newborns (coefficient of correlation r = -0,96, -0,89 respectively). Where high decreased of miRNA-23b associated with death in early life by sepsis. In late sepsis, did observed any correlation between miRNA-23b and death.

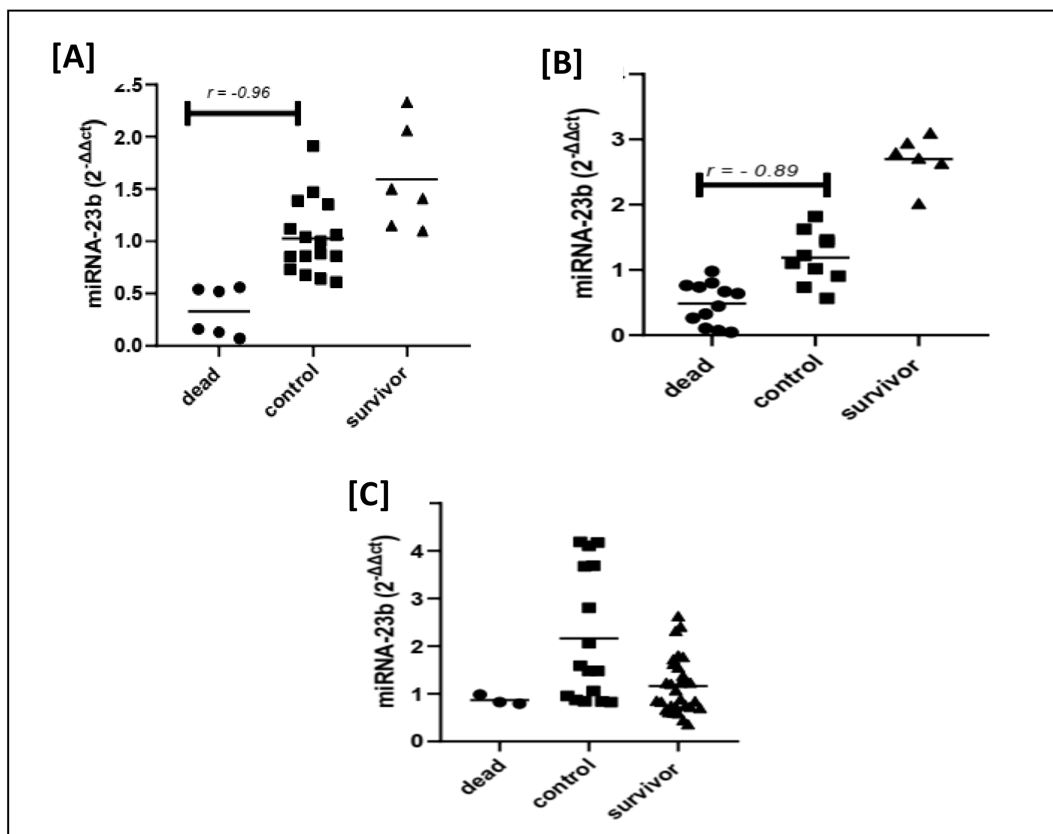

Figure.2. 7 Correlation between miRNA-23b expression and dead newborns with sepsis .A: in early onset sepsis in at-term newborns, B: early onset sepsis in premature newborns, C: late onset sepsis in at-term newborns. Control: patients with negative haemoculture, dead: dead patients with sepsis, survivor: survivor patients with sepsis, r: correlation coefficient

2.6. Results of immunological study (*Ex vivo* model)

In this part of the study, we examined our *ex vivo* model, where the effect of ROS in the translocation of bacteria from the Wharton's jelly to the blood, and the modulation of endothelial cells activation against sepsis.

2.6.1. Effect of $CuSO_4$ and *S. aureus* in NO

The histograms of **Figure 2.9** show that NO concentration at 24-hour was significantly increased in the cells to segment infected alone compared to the control negative ($p < 0,05$ by Mann-Whitney *U* test), the other cultures showing an increase but are not significant **(Figure 2.9, Left panel)**. The 48-hour histogram shows a slightly increased concentration that is not significant between all different culture cells ($p < 0,05$) **(Figure 2.9, Right panel)**.

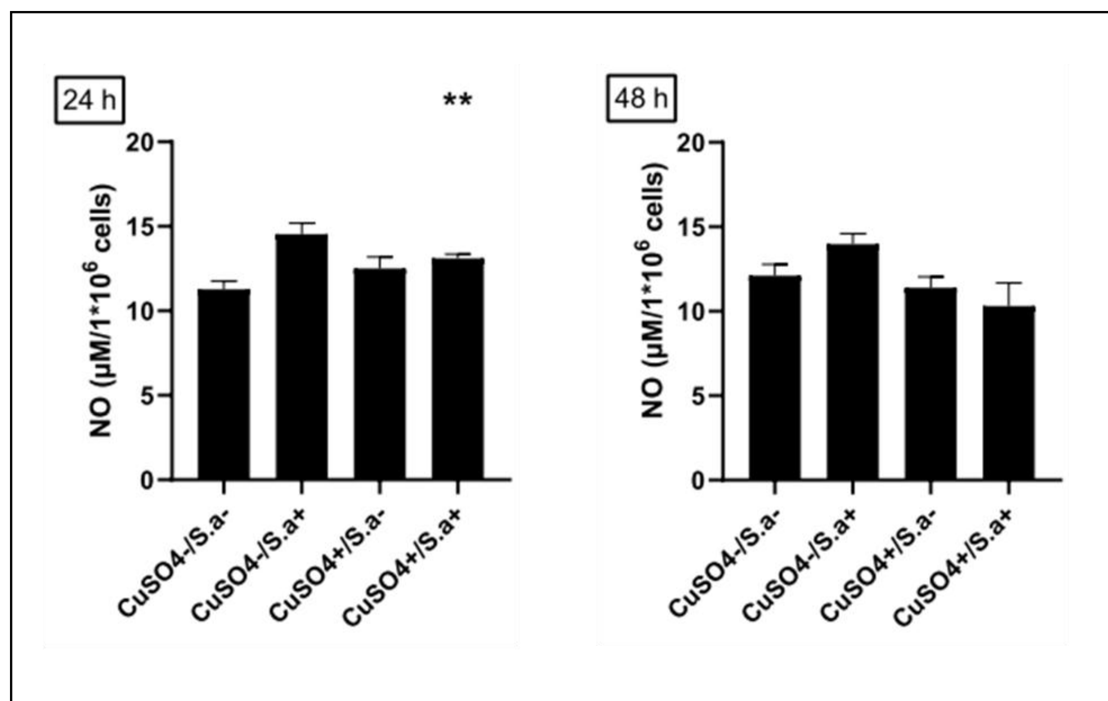

Figure.2. 8 Effect of $CuSO_4$ on functional activities of the endothelial cells *S. aureus* incubation after 24 h and 48 h. NO production assayed by the colorimetric Griess

reaction. H_2O_2 levels measured with highly sensitive spectrophotometric method. The results values correspond a mean and standard error of mean SEM of five independent experiments in each group. H_2O_2: hydrogen peroxide, NO: nitric oxide. $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S.aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.2. $CuSO_4$ and *S. aureus* effect in EC eNOS activity, arginase, eNOS activity-to-arginase activity ratio and NO activity-to-arginase activity ratio

As demonstrated in **Figure 2.10**, the $CuSO_4$ and infection by *S. aureus* significantly upregulate the level of eNOS either in association or separately ($p < 0,05$ by Mann-Whitney *U* test), in the 24-hour culture **(Figure 2.10A, Right panel)**. Conversely in the 48-hour culture there is a mild increase in eNOS levels in cultures $CuSO_4$- / S .a+ and $CuSO_4$ + / S .a-, a slight decrease in the culture $CuSO_4$+ / S .a+ on compared with the control culture **(Figure 2.10 A, Left panel)**.

When we see the histogram of arginase at the 24h, we observe a significant upregulation in cultures $CuSO_4$- / S .a+, $CuSO_4$- / S .a+ and $CuSO_4$+ / S .a+ ($p < 0,05$ by Mann-Whitney *U* test respectively), we also registered a remarkable downregulation in arginase levels in the culture treated by $CuSO_4$ and infected with *S. aureus* compared to the cells in $CuSO_4$+ / S .a- ($p < 0,05$ by Mann-Whitney *U* test). In the 48h culture we notice an increase in the culture infected with S.aureus and in the culture $CuSO_4$+ / S .a+ treated and infected, differently in the condition $CuSO_4$+ / S .a- shows a slight decrease that are not significant. When we compared the culture $CuSO_4$+ / S.a+ with $CuSO_4$+ / S.a-, we found a significant increase in the level of arginase. ($P > 0,05$ by Mann-Whitney *U* test). (For all comparisons, $p < 0,05$ by Kruskal-Wallis test) (**Figure 2.10 B, left and Right panel respectively**).

Furthermore, the ratio of eNOS activity-to-arginase activity decreased significantly in cells extracts infected by S.a and a slowly non-significant decrease in the $CuSO_4$+ / S.a-

in comparison with untreated control in 48 h culture **(Figure 2.10 C)**. For the culture segments treated and infected a significant decrease comparing to $CuSO_4$+ / S .a+ ($p <$ 0,05). Was found in 48 h histogram we show significant decrease in the case of $CuSO_4$- / S.a+, a light decrease in $CuSO_4$+ / S.a-, a greater reduction compared to control negative or in case to present $CuSO_4$ alone has been observed in the culture $CuSO_4$+ / S.a+ **(Figure 2.10 C)**. The NO production to arginase activity ratio were decreased in the cells of piece infected $CuSO_4$- / S.a+, increased significantly in group treated with $CuSO_4$ ($CuSO_4$+ / S.a-) or monitoring with infected $CuSO_4$+ / S.a+ than in controls ($p < 0,05$ by Mann-Whitney *U* test. *p*-Values with Kruskal-Wallis tests were < 0,05 for all comparisons) **(Figure 2.10 D)**.

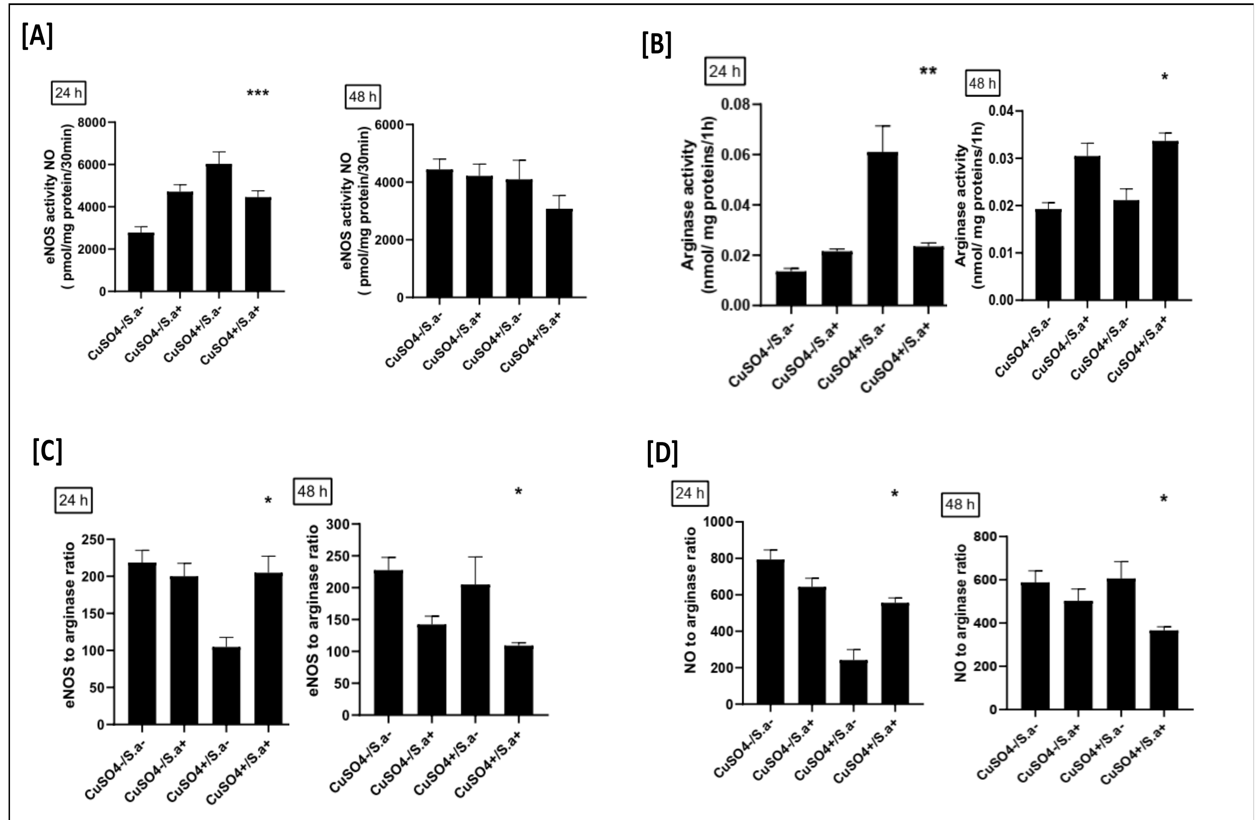

Figure.2. 9 Effect of $CuSO_4$ on eNOS and arginase activity, eNOs activity-to-arginase activity ratio and NO activity-to-arginase activity ratio of the endothelial cells *S. aureus* incubation after 24 h and 48 h. A: Arginase activity levels were determined spectrophotometrically by measurement of the urea concentration after the addition of L-arginine. B: The eNOs activity was determined from NO levels relative to protein concentration. C: eNOs activity-to-arginase activity ratio of the endothelial cells S. aureus incubation after 24 h and 48 h. D: NO activity-to-arginase activity ratio of the endothelial cells S. aureus incubation after 24 h and 48 h. eNOs: endothelial nitric oxide synthase. $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S.aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.3. Effect of $CuSO_4$ and *S. aureus* in catalase activity

Figure 2.11, shows the activity of catalase at 24 and 48 hours. In the 24 h histograms, we observe a significant increase in cells with segments infected with *S. aureus* ($p <$ 0,05 by Mann-Whitney *U* test), in the case of treatment with $CuSO_4$ and infection there is an increase but it not statistically significant. However, this catalase activity decreased significantly in cells treated with $CuSO_4$ only ($p <$ 0,05) (**Figure 2.11, left panel**).

After 48 hours of incubation, the catalase activity in general decreases in different situations so that the expression remains the same at 24 hours. Where at 48h results show that the level of catalase increase significantly in cells from segment infected compared to the negative control ($p < 0,05$ by Mann-Whitney U test), the same case happens for the cells from segment treated by $CuSO_4$ and infected **(Figure 2.11, Right panel)**. The catalase level decreases significantly in $CuSO_4$-treated segment cells ($p < 0,05$). Treatment with $CuSO_4$ and bacteria decreased statistically the catalase activity compared to cells treated with $CuSO_4$ ($p > 0,05$). For all comparisons, $p > 0,01$ per Kruskal-Wallis test **(Figure 2.11)**.

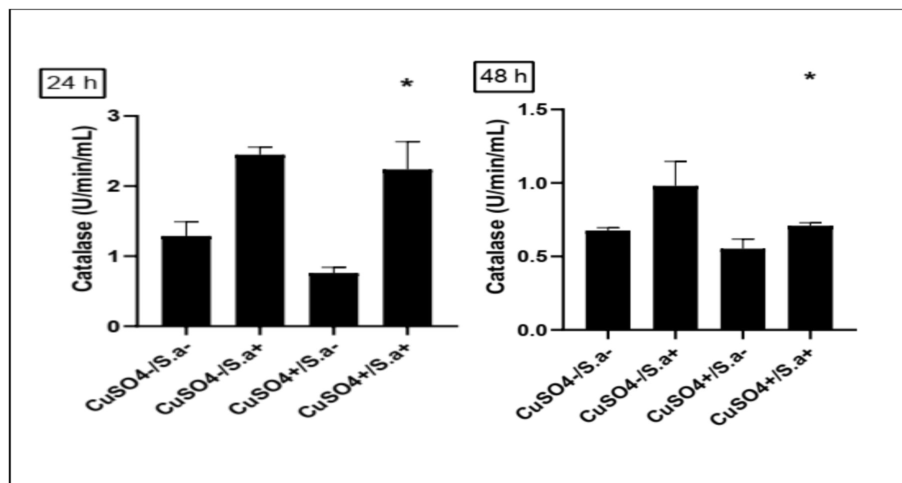

Figure.2. 10 Effect of $CuSO_4$ on catalase activity in the endothelial cells during *S. aureus* incubation. Catalase activity levels determined by spectrophotometrically measurement of the decomposition of H_2O_2. The results values correspond a mean and standard error of mean SEM of four independent experiments in each group. K-W: Kruskal-Wallis test, $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.4. Effect of $CuSO_4$ and *S. aureus* on intracellular calcium levels

We can see in **Figure 2.12** that the calcium ion concentration is increased in the *S. aureus* infection but the highest levels were found when oxidative stress is induced by $CuSO_4$ without infection ($p < 0,05$) **(Figure 2.12, Right panel)**, for the difference between all samples $p < 0,005$ by Kruskal-Wallis test. However, after 48 hours of culture, there is no significant difference registered between all samples **(Figure 2.12, Left panel)**.

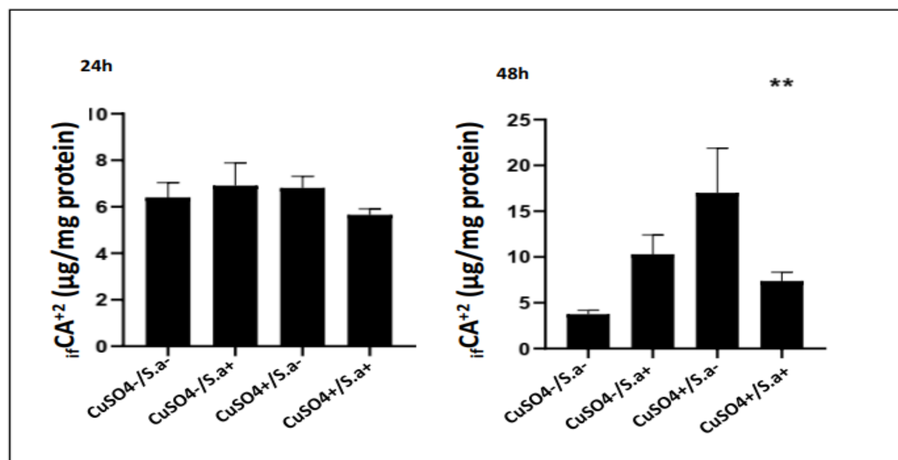

Figure.2. 11 Effect of $CuSO_4$ on intracellular free calcium ion content in the endothelial cells during *S. aureus* incubation. Intracellular free calcium ions levels were biochemically measured by the ortho-cresolphthalein complexone method. The results values correspond a mean of $_{if}Ca^{2+}$ to total proteins and standard error of mean SEM of five independent experiments in each group. K-W: Kruskal-Wallis test, $_{if}Ca^{2+}$: intracellular free calcium ions, $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.5. Effect of $CuSO_4$ and *S. aureus* on the secretion of sICM-1 and E-Selectin

After 24 hours of culture, the sICAM-1 level very slightly decreased in cells extracting from different conditions compared to control negative **(Figure 2.13 A, Left panel)**.

In contrast, after 48 hours of culture, there is a significant increase in different conditions compared to the control (p < 0,05 by Mann-Whitney U test, p < 0,01 by Kruskal-Wallis) **(Figure 2.13 A, Right panel)**. In the 24-hour culture, there were different of expression in E-Selectin histograms between the culture of infected segment $CuSO_4$- / S.a+ and the control. But significantly increased in culture treated with $CuSO_4$ ($CuSO_4$+ / S.a-), and significantly downregulated in the case treated by $CuSO_4$ and infected $CuSO_4$+ / S.a+ (p < 0,05 by Mann-Whitney U test, p < 0,01 by Kruskal-Wallis) **(Figure 2.13 B, Left panel)**.

After 48 hours in culture, the results showed a significant decrease in E-selectin secretion in all conditions compared to the negative control (for all comparison p < 0,05) **(Figure 2.13 B, Right panel)**.

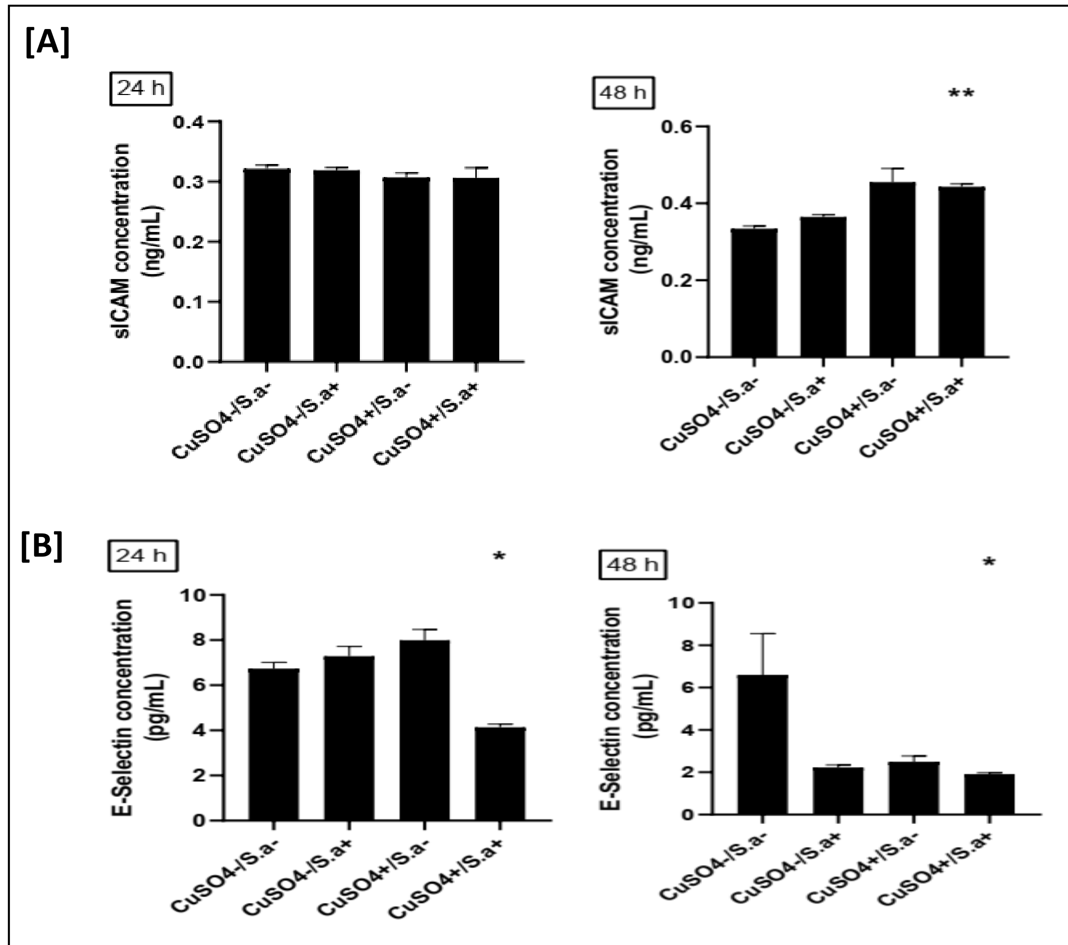

Figure.2. 12 Effect of $CuSO_4$ on sICAM-1 and E-Selectin in the endothelial cells during *S. aureus* incubation. A: the expression of sICAM, and B: the expression of E-Selectin. sICAM-1 and E-Selectin levels were determined by spectrophotometrically using sandwich enzyme-linked immunosorbent assay (ELISA). The results values correspond a mean and standard error of mean SEM of four independent experiments in each group. sICAM-1: soluble intercellular adhesion molecular, $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.6. Effect of $CuSO_4$ and *S. aureus* on the secretion of miRNA-23b

We show in **Figure 2.14**, the results obtained from miRNA-23b in our model. For the 24h culture, the miRNA-23b expression decreases significantly in the infected cord without $CuSO_4$ injection ($p < 0{,}005$).

The miRNA-23b level decreases slightly in the following two cases, treated with $CuSO_4$ without infection or treated with $CuSO_4$ and the injection of *S.aureus* in the Wharton Gelley without any significant **(Figure .2.14, Left panel)**. The histograms at 48h show a significant decrease in the level of miRNA-23b in the supernatant of the HUVEC culture infected without $CuSO_4$ ($p < 0,005$), with a slight decrease with $CuSO_4$ treatment, followed by a significant decrease in the cells in the fourth $CuSO_4$-treated S.aureus infected specimen ($p < 0,005$). For all comparison by KW $p < 0,005$ **(Figure.2.14, Right panel)**.

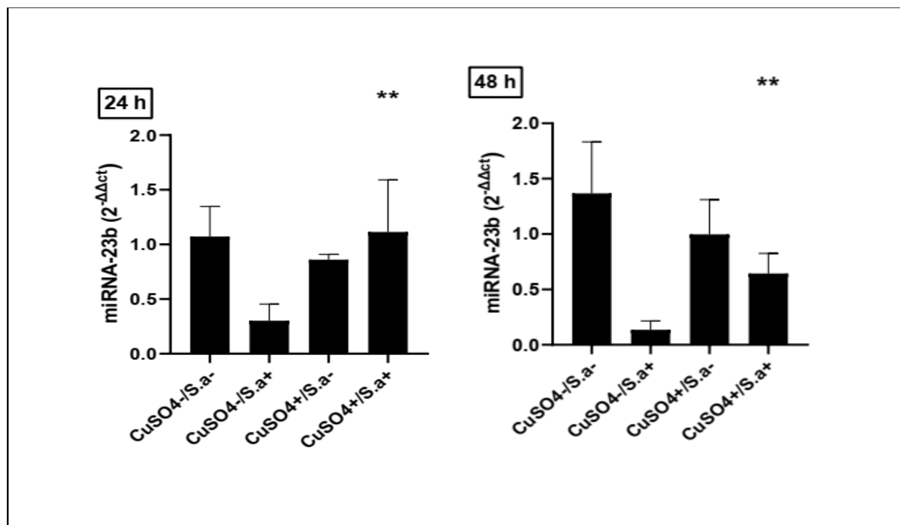

Figure.2. 13 Effect of $CuSO_4$ on miRNA-23b in the endothelial cells during *S. aureus* incubation. miRNA-23b levels were determined by Real-time qPCR, reported as ($2^{-\Delta\Delta CT}$). The results values correspond a mean and standard error of mean SEM of four independent experiments in each group. miRNA-23b: microRNA-23b, $CuSO_4^-$/S.a$^-$: cells from healthy controls not treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^-$/S.a$^+$: cells not treated with $CuSO_4$ and infected by *S. aureus*, $CuSO_4^+$/S.a$^-$: cells treated with $CuSO_4$ and not infected by *S. aureus*, $CuSO_4^+$/S.a$^+$: cells treated with $CuSO_4$ and infected by *S. aureus*

2.6.7. Histological study

The umbilical vessels in the histological pieces formed two arteries and one vein in the four pieces. The umbilical vein is larger with thinner media and fewer smooth muscle cell laminae. In addition, we observed a layer of endothelial cells that lines the internal face of these vessels.

As for the second piece, in which we have injected the bacteria with betamethasone without the stress in the blood level, we noticed the presence of a group of cocci (*S.aureus)* in the laminae of the vein and arteria (**Red circles in Figure 2. 15 (B) and Figure 2. 16 (A, B), respectively**). In Figure 2. 15 (A), we observed the adhesion of lymphocytes to the endothelial cells and Wharton's jelly shows large open spaces between fibers.

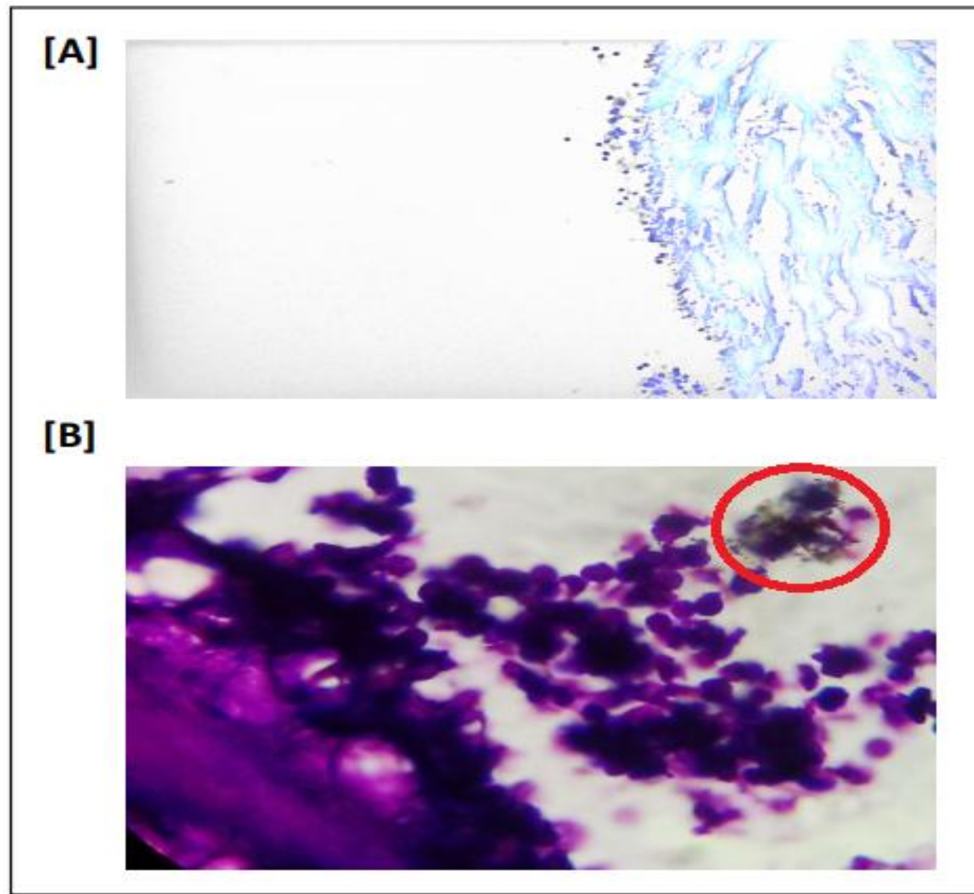

Figure.2. 14 Microscopic observation of intima in the vein of umbilical cord histological study. Effected by microscope optic. The red circle in the figure **B** shows the localization of bacteria inside laminae of the vein

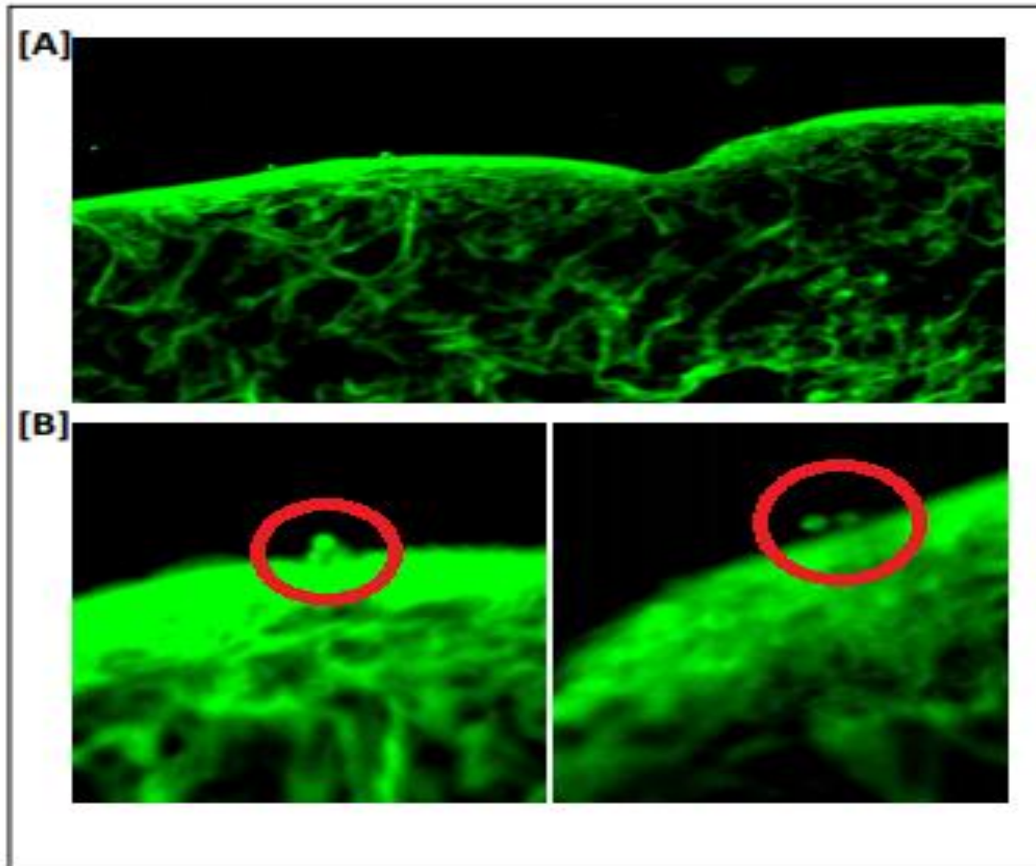

Figure.2. 15 Microscopic observation of intima in the arterial of umbilical cord histological study. Effected by inverted cell imaging fluorescence microscopy station. The red circle shows the localization of bacteria inside laminae of areteria

Chapter 3: Discussion And Conclusion

Chapter 3: Discussion and Conclusion

3. Discussion

Starting with the epidemiological part, in our neonatal department, the EOS was more dominant than LOS. These results can be explained by the difference in age between the two groups as well as the immune system activity of newborns in the first days. A similar result was detected by Atif *et al.*; in (2008) [8]. Inversely in the study to Chabni *et al.;* in (2015) were found LOS more dominant to EOS [34], worked on neonatal nosocomial infection cases in Algeria. In general, EOS is acquired through vertical transmission and therefore associates with organisms that colonize the genital tract. Newborns can be affected by these organisms as they ascend through the amniotic fluid or the birth canal. Invasive infection can occur if the organisms penetrate the skin barrier via the infected fluid inhalation or trans-placental penetration **[108]**.

3.1. Discussion of microbiological study

The main causal microorganism of neonatal sepsis reported were Gram-positive bacteria, particularly the *Staphylococcus spp*. Among the 33 strains of bacteria, 09 strains of CONS were detected, accounting for 27,27 % of the cases. Among Gram-negative bacteria, *K. pneumoniae*. accounted for the highest proportion, 18,18 %, followed by *Stenotrophomonas maltophilia* (9,09 %) that were the case of three babies dead, whereas the overall detection rate was low and basically consistent with that reported in another study [109].

Suggesting the causative pathogens detected in the department remained mainly Gram-positive cocci, especially coagulase-negative *S. hominis* and *S. epidermidis* followed by *K. pneumoniae*. This result is similar to other studies where the Gram-positive bacteria where dominant, the main pathogen differ between the countries [31, 109–111] which might be related to geographical differences or the number of samples affected.

The incidence of birth and fetal mortality are key factors when assessing the level of social protection in a country [112, 113]. In neonatal sepsis, the treatment by antibiotics, where they target the infecting pathogen, but not the inflammatory process, which continues to increase. Therefore, an ideal treatment approach should include antimicrobials and anti-inflammatory drugs to neutralize the rising inflammatory cascade and the resulting "*cytokine storm*" in NS [114]. The immune status of newborns during the perinatal period is different from adults. Neonatal immune responses are generally directed against the generation of Th1-related pro-inflammatory immune responses while favoring Th2-related anti-inflammatory/immunosuppressive response [115]. This process represents an efficient strategy to address the unique challenges of the neonatal period, including maintaining tolerance to maternal antigens in utero and balancing the transition from the sterile intrauterine environment to the antigen-rich outside world [115].

miRNAs have the ability of post-transcriptional gene regulation, which can be done by direct mRNA degradation or the inhibition of their translation [17]. To date, it became obvious that the expression of more than 30 % of human genes is controlled by miRNAs [116].

miRNAs regulate molecular signaling pathways and immune activities [117] and after a pathogenic invasion, a rapid miRNAs production. Thus, miRNA promotes the release of inflammatory factors causing immune hyperactivity, and inducing apoptosis or degrading inflammatory factors that can cause immunosuppression [118, 119]. Biomarkers commonly used in neonatal sepsis have yet to be completely conclusive although they have shown some potential for in vitro diagnosis [120]. Since their discovery, circulating miRNAs in human peripheral serum are used as biomarkers for various types of cancers. The use of miRNAs as diagnostic and prognostic markers has extended to other diseases, including sepsis, however, their role in infectious diseases has rarely been studied [121, 122]. One of the main obstacles in establishing a well-defined link between miRNAs and sepsis is the fact that sepsis can be caused by very different factors; these cause similarities and differences influencing the patient situation itself making sepsis so complicated [123]. For this reason, the association of miRNAs with the diagnosis of sepsis remains controversial [124].

miRNAs from different biological fluids can be used for the early prediction and evaluation of neonatal sepsis, where various miRNAs are downregulated, contributing to the initiation of the immune response to infection [125]. To date, there are no studies on miRNAs in both types of neonatal sepsis, *i.e.*, early and late onset sepsis. Thus far, to the best of our knowledge, there is no study investigating the expression levels of miRNAs in haemoculture from septic patients and their change according to the types of neonatal sepsis.

The neonatal immune response to sepsis depends on the timing of onset, relative pathogens, and developmental age (premature newborns, full-term newborns, infants, *etc.*) [126].

It is markedly different from the immune response in adults because of specific neonatal microbial susceptibility and atopic properties. Differences have also been reported in the regulation of target gene expression by miRNAs in innate immunity [117]. A study of ten immune-regulating miRNAs whose expression was significantly altered more than twice in neonates with sepsis compared to uninfected neonates showed that miRNA expression levels are altered and associated with the regulation of the immune response in neonatal sepsis [125].

In another study, decreased levels of miR-26a have been correlated with upregulation of IL-6 expression in mononuclear blood cells and serum; nevertheless, neither the age of newborns nor the type of sepsis has been specified. Moreover, it has been reported that miR-15a/16 can be used as a potential biomarker for diagnosis and prognosis of neonatal sepsis and that miRNA15a/16 regulation may limit the inflammatory response to LPS [127].

Although the discovery of miRNA-23b is recent [17, 92], intense research has succeeded in showing that it is involved in various physiological and pathophysiological processes. So, it was shown to be an essential moderator of several physiological pathways that regulate the differentiation of multiple cell lines such as keratinocytes, chondrocytes, and skeletal muscle. miRNA-23b regulates also the inflammatory response in several autoimmune diseases through suppressing of pro-inflammatory signaling pathways in resident cells macrophages [86], and plays a critical role in certain pathologies, including acute myocardial infarction (AMI), inflammatory heart diseases, and sepsis-induced cardiac dysfunction [87, 128, 129], diabetic nephropathy [88] and prostate cancer [89].

In sepsis, miRNA-23b has been reported to be downregulated in peripheral mononuclear blood cells (PBMCs) from adult patients and in LPS-induced THP-1 human monocyte cell line, and negatively correlated with the production of pro-inflammatory cytokines. Additionally, increased expression of miRNA-23b has been shown to induce downregulation of pro-inflammatory cytokines production and LPS-stimulated apoptosis [91].

From our side, we showed the possibility of measuring miRNA in general in haemoculture, so that we do not need direct patient blood, even after five hours of incubation until to use it. In addition, we showed that at term birth, neonates with negative haemocultures (control group) showed decreased miRNA-23b levels in the first 72 h of life, which begin to increase after 72 h. Conversely, neonates with a positive blood culture who did not survive the infection always showed down levels of miRNA-23b regardless of whether they were premature or born normally. Additionally, neonates with positive haemoculture that survived infection showed high miRNA-23b during the first 72 h of life, regardless of whether they were born at term or born prematurely. Thus, the increase in miRNA-23b levels during the first 72 h of life in septic neonates may be a potent prognostic factor for survival and a sensitive clinical marker, both in preterm and at term neonates.

It has been shown using an animal model of sepsis that miRNA profiles in $CD8^+$ T cells from adult and neonatal mice were surprisingly similar during infection. However, in absence of infection miRNA levels were different. In particular, marked differences were observed in the expression levels of miRNA-29 and miRNA-130 between adult and neonatal cells before infection. Likewise, changes in the expression of messenger RNA targets have been noted for both miR-29 and miR-130 [130].

In our study, we showed a difference in miRNA-23b expression levels in EOS and LOS. Hence, the expression levels of miRNA-23b increased in EOS patients with positive blood culture and decreased in LOS patients, either in premature or full-term newborns. Nevertheless, we also observed that there is a difference in expression over time in the control group before and after 72 h of birth. This could be due to differences in genome expression patterns in newborns between early- and late-onset sepsis.

Exclusive to newborns, uninfected status and host response to sepsis is significantly affected by the time of birth [85, 131], in which the development of an immune system is a continuous process throughout embryogenesis and into childhood. Hence, the differential expression of miRNAs in neonatal sepsis could be considered a developmental characteristic of the immune response [125]. Then, early and late sepsis responses differ considerably, depending on the postnatal age at the moment of sepsis [132]. By controlling postnatal age in studies of epigenetic changes during neonatal sepsis, we could be able to better understand the immune mechanism in newborns and to identify therapeutic targets. From these results, we suggest the possibility of using miRNA-23b levels as an *in vitro* diagnosis marker, which can be used to differentiate between EOS and LOS. Therefore, the levels of miRNA-23b are upregulated during the first 72 h of life and downregulated over time.

3.2. Discussion of immunological study

Overall, knowledge on neonatal vascular endothelium is much more limited. Neonatal endothelial cells are characterized by their lower expression of adhesion molecules compared to adults, and a reduced capacity to neutralize reactive oxygen species [54].

Similarly, in human umbilical vein endothelial cells (HUVECs) from preterm neonates after LPS stimulation, they noted defective up-regulation of inducible E-selectin, ICAM-1, and VCAM-1 [133]. In any neonatal sepsis study, the models like ours must respond to two concepts: 1) in vitro models to study neonatal vascular endothelium should carefully reproduce all age-specific micro-physiological conditions, such as the quality and origin of plasma, the sub-substrate. 2) interacting immune cells, to more accurately model age-specific biology that is relevant in vivo, thereby improving translational research. Second, even in the context of adult sepsis, most researchers use HUVECs as a model because of their availability and practicality. If we admit that there are differences between neonatal and adult endothelium, most of the results obtained with HUVEC model could more closely reflect neonatal rather than adult physiology [32].

In general, in adults or newborns, endothelial cells constitute the inner wall of a blood vessel and provide an anticoagulant barrier between the vessel wall and the blood [54]. The EC represents not only a selective permeability barrier but also a unique multifunctional cell with critical basal and inducible metabolic and synthetic activities. The EC reacts with physical and chemical stimuli in the circulation and regulates hemostasis, vasomotor tone, immune and inflammatory responses. Injuring, activation, or dysfunction of the endothelial cell are a hallmark of many diseases [51, 134]. To regulate vascular tone, endothelial cells produce vasoactive and vasodilation factors. Where the leading vasodilator factor is nitric oxide, produced by endothelial nitric oxide synthase (eNOS) [135]. Its function is modulated by the intracellular calcium concentration [136, 137].

In general, endothelial dysfunction is associated with a redirection of the actions of the endothelium towards reduced vasodilatation, a pro-inflammatory state, and prothrombotic properties. Free radicals can perturb the NO balance, damaging the endothelium and leaving it too permeable, causing the passage of toxins into body tissues [51]. NO production by ECs is a key defense mechanism against vascular dysfunction across multiple vascular beds, including the pulmonary circulation [138]. In septic pathology, NO plays an important role, since one of its derivatives, peroxynitrite ($ONOO^-$) has bactericidal properties, but it is also toxic to cellular metabolism. Where it's massive production during sepsis is responsible for the macro- and microvascular dysfunction [137]. The production of NO not only by eNOS, but also by inducible NO synthase is one of the main triggers of hypotension in sepsis. However, improvement of sepsis-induced hypotension via NOS inhibitors is not uniformly effective [7].

During severe *S. aureus* infection, the bacteria and their toxins can propagate in the bloodstream by affecting the integrity of endothelial cells, leading to an increase in vascular permeability. In endothelial cells, e.g. *S. aureus* alph-atoxin induces an increase in cytosolic free Ca^{2+} [139]. In our study, and Figure 2.3, we show that the administration of betamethasone significantly neutralizes the inflammatory activity of endothelial cells by increasing NO levels and attenuating pro-inflammatory cytokine levels (Figure.2.7). Subsequently, the translocation of bacteria to the bloodstream is confirmed by haemoculture of blood in the piece injected by bacteria without $CuSO_4$ as can be shown in figures 2.14 and 2.15 of histological study.

Generally, the Glucocorticoids suppress inflammatory gene expression in several cell types and increased susceptibility to infection [140].

The study of Aida *et al.;* in (2004) [141] contradicted our result. They demonstrate the potential of betamethasone to down-regulate eNOS in cultured baboon endothelial cells via glucocorticoid receptor pathways. In another cross-sectional clinical trial, mothers received two doses of betamethasone or placebo administered intramuscularly only 24 hours. After observing these children after 30 years, they concluded that exposure to betamethasone did not affect body size, blood lipids, blood pressure, plasma cortisol, diabetes prevalence, or history of cardiovascular diseases. Therefore, prenatal exposure to betamethasone can lead to insulin resistance in adult offspring [142]. This study lasted for a 30 years and it depends on the indirect effect. Glucocorticoids have already been shown to reduce oxidative stress in the lungs in the case of inflammation, in a lamb model of persistent pulmonary hypertension PPHN Betamethasone reduces oxidative stress and improves the response of pulmonary arteries to vasodilators in lambs [143]. In addition, another study shows that freshly isolated HUVECs were incubated with betamethasone (12 h) before inoculation in the flow chambers. HUVEC pretreated with betamethasone promoted LPS-induced adhesion of adult polymorphonuclear PMNs to the same extent as HUVECs without prior incubation with betamethasone. Furthermore, they suggested that betamethasone does not interfere with neutrophil recruitment, because flow cytometric analysis demonstrated the LPS induced up-regulation of E-selectin and ICAM-1 in HUVEC was independent of prior betamethasone treatment [135]. Another study reported that the dose of 0.2 mg/kg of fetal betamethasone was administered to the rat was chosen to downregulate its effects on fetal mRNA and protein levels [144].

ROS circulating in the bloodstream can interact directly with endothelial cells in the inner lining of blood vessels, increasing intracellular oxidative stress in the EC [145].

To create oxidative stress in the blood, we chose a usually used molecule "sulfate copper". Whereas Cu is an essential trace element that is absorbed, where it plays an important role in the mitochondrial respiratory chain, anti-oxidative defense, and iron metabolism. Some studies suggested that Cu-dependent cell proliferation is mediated through enhanced binding of growth factors to the cell surface, increased secretion of proangiogenic peptides such as FGF1 and IL-1α [146].

On the other hand, Cu deficiency alters the expression of adhesion molecules, such as ICAM-1/VCAM-1 and intravascular adhesion of leukocytes to the activated EC [147]. Cu at a higher level becomes toxic and can catalyze the formation of highly reactive hydroxyl radicals [148]. In (1992), Kishimoto *et al* showed that viability, subsequent growth, morphology, and DNA synthesis were inhibited in a concentration-dependent manner by the addition of copper. The toxic dose of copper starts from 1 µM-100 µM [149]. Incubation of human umbilical vein endothelial cells for 48 hours with 500 µM $CuSO_4$ stimulates cell proliferation. Copper-induced proliferation and migration may suggest a possible mechanism for copper involvement in the angiogenesis process [150]. In an animal model, mice with ischemia where they used only one group, but it was treated three times with 40 µl of $CuSO_4$ (4 µg/ml, once every two days). Immunohistochemical staining results at day 7 post-treatment indicated significantly more CD31 positive endothelial cells in the $CuSO_4$ group than in the saline control group ($P < 0,001$) [151]. In our study, after one hour of injection $CuSO_4$ in the blood, we detected in the cells an increasing in the expression of $_{if}Ca^{+2}$ (Figure. 2.12), and NO (Figure. 2.9), but the expression of H_2O_2 (Figure. 2.9), eNOS, arginase activity (Figure. 2.10) and catalase (Figure. 2.11) decreased.

To clarify its effective role as transition metal cofactor, in the study of the responses of copper-stressed larvae and their macrophages and neutrophils to infection by *Aeromonas hydrophila*. Neutrophils and macrophages in copper-stressed embryos were found to respond very rapidly to infection with *A. hydrophila*, suggesting that copper may enhance the cell responses of neutrophils and macrophages at the bacterially infected locus [152].

In our study, the copper in blood probably plays the same role in improving the cellular responses at the infected bacterial locus. Another study mentioned Cu deficiency affects the intravascular adhesion of leukocytes to activated endothelial cells and the expression of adhesion molecules, such as ICAM-1/VCAM-1. This means Cu has an important role in inflammatory responses involved in innate and adaptive immunity through activation of NF-kB [146]. In addition, *in vivo* macrophages increase the concentration of Cu in the phagosomes via the ATP7A Cu transporter, which increases the bactericidal activity of the phagosome [153].

With increasing the intracellular copper concentration in *S. aureus*, CsoR complexes with Cu^{1+} are released from the DNA and reduce the transcription of copper resistance genes. Probably due to the importance of Cu resistance for *S. aureus* [153]. Sepsis can be prevented experimentally by endothelial cytoprotection by targeting nuclear signaling that mediates inflammation and disrupted metabolism. Endothelial "rheostats", such as miRNAs, regulate endothelial signaling [12].

The miRNA-23b is highly conserved RNA, with less than 0.5 kilobases (kb) of length. In humans, the gene coding miRNA-23b is located on chromosome 9 [154].

miRNA-23b has several roles, which contribute to the regulation of several signal pathways [17].

It has been involved in the prevention of multiple autoimmune diseases through the regulation of inflammatory cytokine signaling pathways [17]. In adult patients with sepsis, the expression of miRNA-23b is related to the manifestation of an inflammatory state and represents a potential tool to evaluate the severity and prognosis of the disease [155]. Evaluation of the levels of miRNA-23b in haemoculture in the first part of our work neonatal, proved the importance of miRNA-23b in favoring the development of sepsis, where we can be used in diagnosis and monitoring marker of sepsis progression. Thus, showed that miRNA-23b levels increased in early onset sepsis, but decreased in late onset sepsis, relatively with negative controls. In both early and late sepsis where the patients die, the levels of miRNA-23b were significantly decreased [57]. In our result (Figure. 2.14), we observed a decrease in miRNA-23b in the cells extracted from the piece where we injected betamethasone and the bacteria without $CuSO_4$, which led to the appearance of bacteria in the blood.

Where miRNA-23b plays an important role in the pathogenesis and progression of sepsis. Downregulation of miRNA-23b inhibits the expression of inflammatory factors including NF-κB, TNF-α, IL-6, ICAM-1, VCAM-1and E-selectin [17]. A single study shows that copper can modulate the expression of miRNAs on the zebrafish olfactory system [156]. Among the limitations of this model, like other models where umbilical cord blood and HUVEC are, although readily available for study, probably our model is more immunotolerant and does not reflect postnatal immune responses; since the neonatal immune system is not clearly demarcated [157].

Conclusion and perspectives

The data obtained in this work show a decrease in the number of patients with neonatal sepsis, but the infectious mortality rate remains high, requiring a larger study than ours. We need to know more about the case of mothers during pregnancy (pregnancy monitoring) since NS is mainly related to the immunity and environment of mothers during pregnancy and after childbirth. The identification of the causal microorganism responsible for sepsis does not show the most frequent strains in the population studied, but also it is the beginning to establish an effective treatment, after the antibiogram and studies of molecular biology.

Our results showed for the first time that haemoculture, is tool of choice to study miRNA changes in neonatal sepsis. Also, the role of miRNA-A23b which is an effective tool for the diagnosis of sepsis, the distinction between EOS and LOS, and the prognosis and worsening of the disease, where it is negatively correlated with death in newborns.

In addition the results obtained from our *ex-vivo* model, show the decrease in the proinflammatory response, and that copper only causes the increase in the proinflammatory and regulated the level of miRNA-23b. These results favored new approaches to "*epi-therapy*". Copper can induce a pro-inflammatory response strengthening the defense mechanism of endothelial cells and indeed could have a potential effect on the behavior of immune cells, and helping to fight against the translocation of bacteria to the blood.

This model needs more studies in order to understand the mechanisms by which ROS in the blood can modify the function of endothelial cells, In addition to the effect of betamethasone in the tissue that led to the translocation of bacteria to the blood.

Therefore, we can summarize the following points as perspectives:

- Monitoring pregnancy and its associated complications are the cornerstone of understanding the mechanism of sepsis in newborns.

- The mechanism of miRNA-23b in sepsis must be elucidated for new approaches to "*epi-therapy*"

- Antibiotic and molecular biology studies of isolated strains have been recommended for effective antibiotic therapy, whether empirical or targeted.

Our *ex-vivo* model requires further studies to elucidate:

- ❖ The mechanism of operation of ROS in the blood.
- ❖ How exactly to act on the EC cells.
- ❖ How the bacteria translocate from tissue to blood.
- ❖ How the immunosuppressant promote bacterial translocation. In addition to this, you need to know more about it.

Chapter 4: Bibliographic

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References

1. Oza S, Lawn JE, Hogan DR, et al (2015) Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000–2013. Bull World Health Organ 93:19–28. https://doi.org/10.2471/BLT.14.139790

2. Kumar SKM, Bhat BV (2016) Distinct mechanisms of the newborn innate immunity. Immunol Lett 173:42–54. https://doi.org/10.1016/j.imlet.2016.03.009

3. Lucignano B, Ranno S, Liesenfeld O, et al (2011) Multiplex PCR Allows Rapid and Accurate Diagnosis of Bloodstream Infections in Newborns and Children with Suspected Sepsis. J Clin Microbiol 49:2252–2258. https://doi.org/10.1128/JCM.02460-10

4. Bhandari V (2014) Effective Biomarkers for Diagnosis of Neonatal Sepsis. J Pediatr Infect Dis Soc 3:234–245. https://doi.org/10.1093/jpids/piu063

5. Panwar C, Kaushik S, Kaushik R, Sood A (2017) Correlation of neonatal and maternal clinico-hematological parameters as predictors of early onset neonatal sepsis. Int J Contemp Pediatr. https://doi.org/10.18203/2349-3291.ijcp20164516

6. Ansari S, Nepal HP, Gautam R, et al (2015) Neonatal Septicemia in Nepal: Early-Onset versus Late-Onset. Int J Pediatr 2015:1–6. https://doi.org/10.1155/2015/379806

7. Cortese F, Scicchitano P, Gesualdo M, et al (2016) Early and Late Infections in Newborns: Where Do We Stand? A Review. Pediatr Neonatol 57:265–273. https://doi.org/10.1016/j.pedneo.2015.09.007

8. Atif ML, Sadaoui F, Bezzaoucha A, et al (2008) Prolongation of Hospital Stay and Additional Costs Due to Nosocomial Bloodstream Infection in an Algerian Neonatal Care Unit. Infect Control Hosp Epidemiol 29:1066–1070. https://doi.org/10.1086/591858

9. Wagstaff JS, Durrant RJ, Newman MG, et al (2019) Antibiotic Treatment of Suspected and Confirmed Neonatal Sepsis Within 28 Days of Birth: A Retrospective Analysis. Front Pharmacol 10:1191. https://doi.org/10.3389/fphar.2019.01191

10. Ng PC (2004) Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed 89:F229-235

11. Shane AL, Sánchez PJ, Stoll BJ (2017) Neonatal sepsis. The Lancet 390:1770–1780. https://doi.org/10.1016/S0140-6736(17)31002-4

12. Hawiger J, Veach RA, Zienkiewicz J (2015) New paradigms in sepsis: from prevention to protection of failing microcirculation. J Thromb Haemost 13:1743–1756. https://doi.org/10.1111/jth.13061

13. Liles WC (2013) Special focus: Endothelial activation/dysfunction. Virulence 4:504–506. https://doi.org/10.4161/viru.26018

14. Huet O, Dupic L, Harrois A, Duranteau J (2011) Oxidative stress and endothelial dysfunction during sepsis. Front Biosci Landmark Ed 16:1986–1995. https://doi.org/10.2741/3835

15. Silva BR, Pernomian L, Bendhack LM (2012) Contribution of oxidative stress to endothelial dysfunction in hypertension. Front Physiol 3:. https://doi.org/10.3389/fphys.2012.00441

16. Peters K (2003) Molecular basis of endothelial dysfunction in sepsis. Cardiovasc Res 60:49–57. https://doi.org/10.1016/S0008-6363(03)00397-3

17. Wu M, Gu J-T, Yi B, et al (2015) microRNA-23b regulates the expression of inflammatory factors in vascular endothelial cells during sepsis. Exp Ther Med 9:1125–1132. https://doi.org/10.3892/etm.2015.2224

18. Lenkala D, LaCroix B, Gamazon ER, et al (2014) The impact of microRNA expression on cellular proliferation. Hum Genet 133:931–938. https://doi.org/10.1007/s00439-014-1434-4

19. Reinhart K, Bauer M, Riedemann NC, Hartog CS (2012) New Approaches to Sepsis: Molecular Diagnostics and Biomarkers. Clin Microbiol Rev 25:609–634. https://doi.org/10.1128/CMR.00016-12

20. Wynn JL (2016) Defining neonatal sepsis: Curr Opin Pediatr 28:135–140. https://doi.org/10.1097/MOP.0000000000000315

21. Ershad M, Mostafa A, Dela Cruz M, Vearrier D (2019) Neonatal Sepsis. Curr Emerg Hosp Med Rep 7:83–90. https://doi.org/10.1007/s40138-019-00188-z

22. Bone RC, Balk RA, Cerra FB, et al (1992) Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. Chest 101:1644–1655. https://doi.org/10.1378/chest.101.6.1644

23. Goldstein B, Giroir B, Randolph A (2005) International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics*: Pediatr Crit Care Med 6:2–8. https://doi.org/10.1097/01.PCC.0000149131.72248.E6

24. Seale AC, Blencowe H, Manu AA, et al (2014) Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. Lancet Infect Dis 14:731–741. https://doi.org/10.1016/S1473-3099(14)70804-7

25. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD (2014) Early-Onset Neonatal Sepsis. Clin Microbiol Rev 27:21–47. https://doi.org/10.1128/CMR.00031-13

26. Benirschke K (1960) Routes and Types of Infection in the Fetus and the Newborn. Arch Pediatr Adolesc Med 99:714. https://doi.org/10.1001/archpedi.1960.02070030716003

27. Manandhar SR, Manandhar DS (2017) Use of antibiotics in newborns with maternal history of premature rupture of membrane. J Kathmandu Med Coll 5:3–10. https://doi.org/10.3126/jkmc.v5i1.18258

28. Mukhopadhyay S, Puopolo KM (2012) Risk Assessment in Neonatal Early Onset Sepsis. Semin Perinatol 36:408–415. https://doi.org/10.1053/j.semperi.2012.06.002

29. Shah BA, Padbury JF (2014) Neonatal sepsis: An old problem with new insights. Virulence 5:170–178. https://doi.org/10.4161/viru.26906

30. Dong Y, Speer CP (2015) Late-onset neonatal sepsis: recent developments. Arch Dis Child - Fetal Neonatal Ed 100:F257–F263. https://doi.org/10.1136/archdischild-2014-306213

31. Vergnano S, Buttery J, Cailes B, et al (2016) Neonatal infections: Case definition and guidelines for data collection, analysis, and presentation of immunisation safety data. Vaccine 34:6038–6046. https://doi.org/10.1016/j.vaccine.2016.03.046

32. Zhou B, Liu X, Wu J-B, et al (2016) Clinical and microbiological profile of babies born with risk of neonatal sepsis. Exp Ther Med 12:3621–3625. https://doi.org/10.3892/etm.2016.3836

33. Odabasi IO, Bulbul A (2020) Neonatal Sepsis. Sisli Etfal Hastan Tip Bul 54:142–158. https://doi.org/10.14744/SEMB.2020.00236

34. Chabni N, Regagba D, Meguenni K, et al (2015) Facteurs de risque de l’infection nosocomiale au niveau du service de néonatologie polyvalente de l’établissement hospitalier spécialisé mère-enfant de Tlemcen à l’Ouest algérien, « étude cas-témoins ». J Pédiatrie Puériculture 28:71–79. https://doi.org/10.1016/j.jpp.2015.02.006

35. Puopolo KM, Draper D, Wi S, et al (2011) Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. Pediatrics 128:e1155-1163. https://doi.org/10.1542/peds.2010-3464

36. Polin RA, Committee on Fetus and Newborn (2012) Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics 129:1006–1015. https://doi.org/10.1542/peds.2012-0541

37. Yu JC, Khodadadi H, Malik A, et al (2018) Innate Immunity of Neonates and Infants. Front Immunol 9:1759. https://doi.org/10.3389/fimmu.2018.01759

38. Kollmann TR, Kampmann B, Mazmanian SK, et al (2017) Protecting the Newborn and Young Infant from Infectious Diseases: Lessons from Immune Ontogeny. Immunity 46:350–363. https://doi.org/10.1016/j.immuni.2017.03.009

39. Palmer AC (2011) Nutritionally mediated programming of the developing immune system. Adv Nutr Bethesda Md 2:377–395. https://doi.org/10.3945/an.111.000570

40. Restori KH, Srinivasa BT, Ward BJ, Fixman ED (2018) Neonatal Immunity, Respiratory Virus Infections, and the Development of Asthma. Front Immunol 9:1249. https://doi.org/10.3389/fimmu.2018.01249

41. Basha S, Surendran N, Pichichero M (2014) Immune responses in neonates. Expert Rev Clin Immunol 10:1171–1184. https://doi.org/10.1586/1744666X.2014.942288

42. Raymond SL, Stortz JA, Mira JC, et al (2017) Immunological Defects in Neonatal Sepsis and Potential Therapeutic Approaches. Front Pediatr 5:. https://doi.org/10.3389/fped.2017.00014

43. Nielsen SCA, Boyd SD (2019) New technologies and applications in infant B cell immunology. Curr Opin Immunol 57:53–57. https://doi.org/10.1016/j.coi.2018.12.005

44. Zaghouani H, Hoeman CM, Adkins B (2009) Neonatal immunity: faulty T-helpers and the shortcomings of dendritic cells. Trends Immunol 30:585–591. https://doi.org/10.1016/j.it.2009.09.002

45. Joyner JL, Augustine NH, Taylor KA, et al (2000) Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon-gamma mRNA accumulation and protein secretion. J Infect Dis 182:974–977. https://doi.org/10.1086/315796

46. Maródi L (2006) Neonatal Innate Immunity to Infectious Agents. Infect Immun 74:1999–2006. https://doi.org/10.1128/IAI.74.4.1999-2006.2006

47. Hill HR, Augustine NH, Jaffe HS (1991) Human recombinant interferon gamma enhances neonatal polymorphonuclear leukocyte activation and movement, and increases free intracellular calcium. J Exp Med 173:767–770. https://doi.org/10.1084/jem.173.3.767

48. Baudin B, Bruneel A, Bosselut N, Vaubourdolle M (2007) A protocol for isolation and culture of human umbilical vein endothelial cells. Nat Protoc 2:481–485. https://doi.org/10.1038/nprot.2007.54

49. Pries AR, Kuebler WM (2006) Normal Endothelium. In: Moncada S, Higgs A (eds) The Vascular Endothelium I. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1–40

50. Krüger-Genge, Blocki, Franke, Jung (2019) Vascular Endothelial Cell Biology: An Update. Int J Mol Sci 20:4411. https://doi.org/10.3390/ijms20184411

51. Rajendran P, Rengarajan T, Thangavel J, et al (2013) The Vascular Endothelium and Human Diseases. Int J Biol Sci 9:1057–1069. https://doi.org/10.7150/ijbs.7502

52. Dejana E, Orsenigo F, Lampugnani MG (2008) The role of adherens junctions and VE-cadherin in the control of vascular permeability. J Cell Sci 121:2115–2122. https://doi.org/10.1242/jcs.017897

53. Lorant DE, Li W, Tabatabaei N, et al (1999) P-selectin expression by endothelial cells is decreased in neonatal rats and human premature infants. Blood 94:600–609

54. Pietrasanta C, Pugni L, Ronchi A, et al (2019) Vascular Endothelium in Neonatal Sepsis: Basic Mechanisms and Translational Opportunities. Front Pediatr 7:340. https://doi.org/10.3389/fped.2019.00340

55. Breitenbach M, Eckl P (2015) Introduction to Oxidative Stress in Biomedical and Biological Research. Biomolecules 5:1169–1177. https://doi.org/10.3390/biom5021169

56. Lushchak VI (2014) Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 224:164–175. https://doi.org/10.1016/j.cbi.2014.10.016

57. Pizzino G, Irrera N, Cucinotta M, et al (2017) Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev 2017:1–13. https://doi.org/10.1155/2017/8416763

58. Li YR, Trush M (2016) Defining ROS in Biology and Medicine. React Oxyg Species 1:. https://doi.org/10.20455/ros.2016.803

59. Lin L-S, Wang J-F, Song J, et al (2019) Cooperation of endogenous and exogenous reactive oxygen species induced by zinc peroxide nanoparticles to enhance oxidative stress-based cancer therapy. Theranostics 9:7200–7209. https://doi.org/10.7150/thno.39831

60. Liguori I, Russo G, Curcio F, et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757–772. https://doi.org/10.2147/CIA.S158513

61. Widlansky ME, Gutterman DD (2011) Regulation of endothelial function by mitochondrial reactive oxygen species. Antioxid Redox Signal 15:1517–1530. https://doi.org/10.1089/ars.2010.3642

62. A. R, I. M, J. J, et al (2012) Oxidative Stress and Antioxidants: Preterm Birth and Preterm Infants. In: Morrison J (ed) Preterm Birth - Mother and Child. InTech

63. Biswal S, Remick DG (2007) Sepsis: Redox Mechanisms and Therapeutic Opportunities. Antioxid Redox Signal 9:1959–1962. https://doi.org/10.1089/ars.2007.1808

64. de Vega JMA, Díaz J, Serrano E, Carbonell LF (2000) Plasma redox status relates to severity in critically ill patients: Crit Care Med 28:1812–1814. https://doi.org/10.1097/00003246-200006000-00021

65. Barichello T, Fortunato JJ, Vitali ÂM, et al (2006) Oxidative variables in the rat brain after sepsis induced by cecal ligation and perforation: Crit Care Med 34:886–889. https://doi.org/10.1097/01.CCM.0000201880.50116.12

66. Hoesel LM, Gao H, Ward PA (2006) New Insights into Cellular Mechanisms During Sepsis. Immunol Res 34:133–142. https://doi.org/10.1385/IR:34:2:133

67. Bosmann M, Ward PA (2013) The inflammatory response in sepsis. Trends Immunol 34:129–136. https://doi.org/10.1016/j.it.2012.09.004

68. Noubade R, Wong K, Ota N, et al (2014) NRROS negatively regulates reactive oxygen species during host defence and autoimmunity. Nature 509:235–239. https://doi.org/10.1038/nature13152

69. Incalza MA, D'Oria R, Natalicchio A, et al (2018) Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascul Pharmacol 100:1–19. https://doi.org/10.1016/j.vph.2017.05.005

70. Fan LM, Douglas G, Bendall JK, et al (2014) Endothelial Cell–Specific Reactive Oxygen Species Production Increases Susceptibility to Aortic Dissection. Circulation 129:2661–2672. https://doi.org/10.1161/CIRCULATIONAHA.113.005062

71. Shabalina SA, Koonin EV (2008) Origins and evolution of eukaryotic RNA interference. Trends Ecol Evol 23:578–587. https://doi.org/10.1016/j.tree.2008.06.005

72. Muljo SA, Kanellopoulou C, Aravind L (2010) MicroRNA targeting in mammalian genomes: genes and mechanisms. Wiley Interdiscip Rev Syst Biol Med 2:148–161. https://doi.org/10.1002/wsbm.53

73. Dexheimer PJ, Cochella L (2020) MicroRNAs: From Mechanism to Organism. Front Cell Dev Biol 8:409. https://doi.org/10.3389/fcell.2020.00409

74. Liang Y, Ridzon D, Wong L, Chen C (2007) Characterization of microRNA expression profiles in normal human tissues. BMC Genomics 8:166. https://doi.org/10.1186/1471-2164-8-166

75. Ludwig N, Leidinger P, Becker K, et al (2016) Distribution of miRNA expression across human tissues. Nucleic Acids Res 44:3865–3877. https://doi.org/10.1093/nar/gkw116

76. Tili E, Michaille J-J, Croce CM (2013) MicroRNAs play a central role in molecular dysfunctions linking inflammation with cancer. Immunol Rev 253:167–184. https://doi.org/10.1111/imr.12050

77. Li S, Patel DJ (2016) Drosha and Dicer: Slicers cut from the same cloth. Cell Res 26:511–512. https://doi.org/10.1038/cr.2016.19

78. Hashemian SM, Pourhanifeh MH, Fadaei S, et al (2020) Non-coding RNAs and Exosomes: Their Role in the Pathogenesis of Sepsis. Mol Ther - Nucleic Acids 21:51–74. https://doi.org/10.1016/j.omtn.2020.05.012

79. Haneklaus M, Gerlic M, O'Neill L a. J, Masters SL (2013) miR-223: infection, inflammation and cancer. J Intern Med 274:215–226. https://doi.org/10.1111/joim.12099

80. Yuan X, Berg N, Lee JW, et al (2018) MicroRNA miR-223 as regulator of innate immunity. J Leukoc Biol 104:515–524. https://doi.org/10.1002/JLB.3MR0218-079R

81. Shukla GC, Singh J, Barik S (2011) MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. Mol Cell Pharmacol 3:83–92

82. Zhang T-N, Li D, Xia J, et al (2017) Non-coding RNA: a potential biomarker and therapeutic target for sepsis. Oncotarget 8:91765–91778. https://doi.org/10.18632/oncotarget.21766

83. Cook J, Bennett PR, Kim SH, et al (2019) First Trimester Circulating MicroRNA Biomarkers Predictive of Subsequent Preterm Delivery and Cervical Shortening. Sci Rep 9:5861. https://doi.org/10.1038/s41598-019-42166-1

84. Rodil-Garcia P, Arellanes-Licea E, Montoya-Contreras A, Salazar-Olivo L (2017) Analysis of MicroRNA Expression in Newborns with Differential Birth Weight Using Newborn Screening Cards. Int J Mol Sci 18:2552. https://doi.org/10.3390/ijms18122552

85. Wynn JL, Guthrie SO, Wong HR, et al (2015) Postnatal Age Is a Critical Determinant of the Neonatal Host Response to Sepsis. Mol Med 21:496–504. https://doi.org/10.2119/molmed.2015.00064

86. Bordon Y (2012) MicroRNA-23b keeps TABs on tissue inflammation. Nat Rev Immunol 12:475–475. https://doi.org/10.1038/nri3250

87. Grossi I, Salvi A, Baiocchi G, et al (2018) Functional Role of microRNA-23b-3p in Cancer Biology. MicroRNA 7:156–166. https://doi.org/10.2174/2211536607666180629155025

88. Zhao B, Li H, Liu J, et al (2016) MicroRNA-23b Targets Ras GTPase-Activating Protein SH3 Domain-Binding Protein 2 to Alleviate Fibrosis and Albuminuria in Diabetic Nephropathy. J Am Soc Nephrol 27:2597–2608. https://doi.org/10.1681/ASN.2015030300

89. Pimenta RC, Viana NI, Amaral GQ, et al (2018) MicroRNA-23b and microRNA-27b plus flutamide treatment enhances apoptosis rate and decreases CCNG1 expression in a castration-resistant prostate cancer cell line. Tumor Biol 40:101042831880301. https://doi.org/10.1177/1010428318803011

90. Zhang H, Caudle Y, Shaikh A, et al (2018) Inhibition of microRNA-23b prevents polymicrobial sepsis-induced cardiac dysfunction by modulating TGIF1 and

PTEN. Biomed Pharmacother 103:869–878. https://doi.org/10.1016/j.biopha.2018.04.092

91. Zhang W, Lu F, Xie Y, et al (2019) miR-23b Negatively Regulates Sepsis-Induced Inflammatory Responses by Targeting ADAM10 in Human THP-1 Monocytes. Mediators Inflamm 2019:1–13. https://doi.org/10.1155/2019/5306541

92. Ou H, Xiao X, Jiang Y, et al (2018) Expression of microRNA-23b in patients with sepsis and its effect on leukocytes and the expression of E-selectin and ICAM-1. Exp Ther Med. https://doi.org/10.3892/etm.2018.6759

93. Singer M, Deutschman CS, Seymour CW, et al (2016) The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 315:801. https://doi.org/10.1001/jama.2016.0287

94. Horan TC, Andrus M, Dudeck MA (2008) CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 36:309–332. https://doi.org/10.1016/j.ajic.2008.03.002

95. Omoregie R, Egbe CA, Dirisu J, Ogefere HO (2013) Microbiology of neonatal septicemia in a tertiary hospital in Benin City, Nigeria. Biomark Genomic Med 5:142–146. https://doi.org/10.1016/j.bgm.2013.06.001

96. Loucif L, Bendjama E, Gacemi-Kirane D, Rolain J-M (2014) Rapid identification of Streptomyces isolates by MALDI-TOF MS. Microbiol Res 169:940–947. https://doi.org/10.1016/j.micres.2014.04.004

97. Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

98. Liebl AL, Martin II LB (2009) Simple quantification of blood and plasma antimicrobial capacity using spectrophotometry: *Antimicrobial capacity and spectrophotometry*. Funct Ecol 23:1091–1096. https://doi.org/10.1111/j.1365-2435.2009.01592.x

99. Gordon Z, Glaubach L, Elad D, et al (2016) Ex Vivo Human Placental Perfusion Model for Analysis of Fetal Circulation in the Chorionic Plate. J Ultrasound Med 35:553–560. https://doi.org/10.7863/ultra.15.04080

100. Marin V, Kaplanski G, Grès S, et al (2001) Endothelial cell culture: protocol to obtain and cultivate human umbilical endothelial cells. J Immunol Methods 254:183–190. https://doi.org/10.1016/S0022-1759(01)00408-2

101. Rabini RA, Tesei M, Galeazzi T, et al (1999) Increased susceptibility to peroxidation of VLDL from non-insulin-dependent diabetic patients: a possible correlation with fatty acid composition. Mol Cell Biochem 199:63–67

102. Weidensdorfer M, Chae JI, Makobe C, et al (2016) Analysis of Endothelial Adherence of Bartonella henselae and Acinetobacter baumannii Using a Dynamic

Human *Ex Vivo* Infection Model. Infect Immun 84:711–722. https://doi.org/10.1128/IAI.01502-15

103. Davis J, Crampton SP, Hughes CCW (2007) Isolation of Human Umbilical Vein Endothelial Cells (HUVEC). J Vis Exp. https://doi.org/10.3791/183

104. Aebi H (1974) Catalase. In: Methods of Enzymatic Analysis. Elsevier, pp 673–684

105. Aribi M, Meziane W, Habi S, et al (2015) Macrophage Bactericidal Activities against Staphylococcus aureus Are Enhanced In Vivo by Selenium Supplementation in a Dose-Dependent Manner. PLOS ONE 10:e0135515. https://doi.org/10.1371/journal.pone.0135515

106. Olsen CH (2003) Review of the use of statistics in infection and immunity. Infect Immun 71:6689–6692. https://doi.org/10.1128/iai.71.12.6689-6692.2003

107. Başustaoğlu A, Süzük Yıldız S, Mumcuoğlu İ, et al (2019) [Evaluation of blood culture practices: Use of system (Epicenter) data]. Mikrobiyol Bul 53:12–21. https://doi.org/10.5578/mb.67782

108. Jefferies AL (2017) La prise en charge des nouveau-nés à terme à risque de sepsis bactérien d'apparition précoce. Paediatr Child Health 22:229–235. https://doi.org/10.1093/pch/pxx024

109. Dong H, Cao H, Zheng H (2017) Pathogenic bacteria distributions and drug resistance analysis in 96 cases of neonatal sepsis. BMC Pediatr 17:44. https://doi.org/10.1186/s12887-017-0789-9

110. Havey TC, Fowler RA, Daneman N (2011) Duration of antibiotic therapy for bacteremia: a systematic review and meta-analysis. Crit Care 15:R267. https://doi.org/10.1186/cc10545

111. Zhou L, Ma X, Gao W, et al (2012) Molecular characteristics of erythromycin-resistant Streptococcus pneumoniae from pediatric patients younger than five years in Beijing, 2010. BMC Microbiol 12:228. https://doi.org/10.1186/1471-2180-12-228

112. Say L, Souza JP, Pattinson RC (2009) Maternal near miss – towards a standard tool for monitoring quality of maternal health care. Best Pract Res Clin Obstet Gynaecol 23:287–296. https://doi.org/10.1016/j.bpobgyn.2009.01.007

113. Gonzalez RM, Gilleskie D (2017) Infant Mortality Rate as a Measure of a Country's Health: A Robust Method to Improve Reliability and Comparability. Demography 54:701–720. https://doi.org/10.1007/s13524-017-0553-7

114. Nedeva C, Menassa J, Puthalakath H (2019) Sepsis: Inflammation Is a Necessary Evil. Front Cell Dev Biol 7:108. https://doi.org/10.3389/fcell.2019.00108

115. Kollmann TR, Levy O, Montgomery RR, Goriely S (2012) Innate Immune Function by Toll-like Receptors: Distinct Responses in Newborns and the Elderly. Immunity 37:771–783. https://doi.org/10.1016/j.immuni.2012.10.014

116. Bartel DP (2004) MicroRNAs. Cell 116:281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

117. Yu H-R, Huang L-H, Li S-C (2018) Roles of microRNA in the immature immune system of neonates. Cancer Lett 433:99–106. https://doi.org/10.1016/j.canlet.2018.06.014

118. Li YC, Chen Y, Liu W, Thadhani R (2014) MicroRNA-mediated mechanism of vitamin D regulation of innate immune response. J Steroid Biochem Mol Biol 144:81–86. https://doi.org/10.1016/j.jsbmb.2013.09.014

119. Chen C-Z, Schaffert S, Fragoso R, Loh C (2013) Regulation of immune responses and tolerance: the microRNA perspective. Immunol Rev 253:112–128. https://doi.org/10.1111/imr.12060

120. Kingsley Manoj Kumar S, Vishnu Bhat B (2015) Current challenges and future perspectives in neonatal sepsis. Pediatr Infect Dis 7:41–46. https://doi.org/10.1016/j.pid.2015.10.005

121. Wang H, Zhang P, Chen W, et al (2013) Characterization and Identification of Novel Serum MicroRNAs in Sepsis Patients With Different Outcomes: Shock 39:480–487. https://doi.org/10.1097/SHK.0b013e3182940cb8

122. Wang H, Zhang P, Chen W, et al (2012) Four serum microRNAs identified as diagnostic biomarkers of sepsis: J Trauma Acute Care Surg 73:850–854. https://doi.org/10.1097/TA.0b013e31825a7560

123. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, et al (2011) The Pathogenesis of Sepsis. Annu Rev Pathol Mech Dis 6:19–48. https://doi.org/10.1146/annurev-pathol-011110-130327

124. Zhang W, Jia J, Liu Z, et al (2019) Circulating microRNAs as biomarkers for Sepsis secondary to pneumonia diagnosed via Sepsis 3.0. BMC Pulm Med 19:93. https://doi.org/10.1186/s12890-019-0836-4

125. Chen J, Jiang S, Cao Y, Yang Y (2014) Altered miRNAs Expression Profiles and Modulation of Immune Response Genes and Proteins During Neonatal Sepsis. J Clin Immunol 34:340–348. https://doi.org/10.1007/s10875-014-0004-9

126. Sweeney TE, Wynn JL, Cernada M, et al (2018) Validation of the Sepsis MetaScore for Diagnosis of Neonatal Sepsis. J Pediatr Infect Dis Soc 7:129–135. https://doi.org/10.1093/jpids/pix021

127. Wang X, Wang X, Liu X, et al (2015) miR-15a/16 are upreuglated in the serum of neonatal sepsis patients and inhibit the LPS-induced inflammatory pathway. Int J Clin Exp Med 8:5683–5690

128. Mirna M, Paar V, Rezar R, et al (2019) MicroRNAs in Inflammatory Heart Diseases and Sepsis-Induced Cardiac Dysfunction: A Potential Scope for the Future? Cells 8:. https://doi.org/10.3390/cells8111352

129. Zhang J, Li Y, Zhao Q (2018) Circulating miR-23b as a Novel Biomarker for Early Risk Stratification After ST-Elevation Myocardial Infarction. Med Sci Monit 24:1517–1523. https://doi.org/10.12659/MSM.908060

130. Wissink EM, Smith NL, Spektor R, et al (2015) MicroRNAs and Their Targets Are Differentially Regulated in Adult and Neonatal Mouse CD8+ T Cells. Genetics 201:1017–1030. https://doi.org/10.1534/genetics.115.179176

131. Raymond SL, Stortz JA, Mira JC, et al (2017) Immunological Defects in Neonatal Sepsis and Potential Therapeutic Approaches. Front Pediatr 5:. https://doi.org/10.3389/fped.2017.00014

132. Ng S, Strunk T, Jiang P, et al (2018) Precision Medicine for Neonatal Sepsis. Front Mol Biosci 5:70. https://doi.org/10.3389/fmolb.2018.00070

133. Wisgrill L, Muck M, Wessely I, et al (2018) Endothelial cells of extremely premature infants display impaired immune response after proinflammatory stimulation. Pediatr Res 83:128–134. https://doi.org/10.1038/pr.2017.202

134. Sumpio BE, Timothy Riley J, Dardik A (2002) Cells in focus: endothelial cell. Int J Biochem Cell Biol 34:1508–1512. https://doi.org/10.1016/S1357-2725(02)00075-4

135. Nussbaum C, Gloning A, Pruenster M, et al (2013) Neutrophil and endothelial adhesive function during human fetal ontogeny. J Leukoc Biol 93:175–184. https://doi.org/10.1189/jlb.0912468

136. Declercq M, Treps L, Carmeliet P, Witters P (2019) The role of endothelial cells in cystic fibrosis. J Cyst Fibros S1569199319308264. https://doi.org/10.1016/j.jcf.2019.07.005

137. Besnier E, Delile E, Coquerel D, Tamion F (2015) Les voies du monoxyde d’azote dans le sepsis. Réanimation 24:191–200. https://doi.org/10.1007/s13546-015-1044-8

138. Suresh K, Shimoda LA (2017) Endothelial Cell Reactive Oxygen Species and Ca2+ Signaling in Pulmonary Hypertension. In: Wang Y-X (ed) Pulmonary Vasculature Redox Signaling in Health and Disease. Springer International Publishing, Cham, pp 299–314

139. Kwak Y-K, Vikström E, Magnusson K-E, et al (2012) The Staphylococcus aureus Alpha-Toxin Perturbs the Barrier Function in Caco-2 Epithelial Cell Monolayers by Altering Junctional Integrity. Infect Immun 80:1670–1680. https://doi.org/10.1128/IAI.00001-12

140. Clayton SA, Jones SW, Kurowska-Stolarska M, Clark AR (2018) The role of microRNAs in glucocorticoid action. J Biol Chem 293:1865–1874. https://doi.org/10.1074/jbc.R117.000366

141. Aida K, Shi Q, Wang J, et al (2004) The effects of betamethasone (BM) on endothelial nitric oxide synthase (eNOS) expression in adult baboon femoral arterial endothelial cells. J Steroid Biochem Mol Biol 91:219–224. https://doi.org/10.1016/j.jsbmb.2004.05.004

142. Dalziel SR, Walker NK, Parag V, et al (2005) Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. The Lancet 365:1856–1862. https://doi.org/10.1016/S0140-6736(05)66617-2

143. Chandrasekar I, Eis A, Konduri GG (2008) Betamethasone Attenuates Oxidant Stress in Endothelial Cells from Fetal Lambs with Persistent Pulmonary Hypertension. Pediatr Res 63:67–72. https://doi.org/10.1203/PDR.0b013e31815b43ee

144. Kim YE, Park WS, Sung DK, et al (2019) Antenatal betamethasone enhanced the detrimental effects of postnatal dexamethasone on hyperoxic lung and brain injuries in newborn rats. PLOS ONE 14:e0221847. https://doi.org/10.1371/journal.pone.0221847

145. Montorfano I, Becerra A, Cerro R, et al (2014) Oxidative stress mediates the conversion of endothelial cells into myofibroblasts via a TGF-β1 and TGF-β2-dependent pathway. Lab Invest 94:1068–1082. https://doi.org/10.1038/labinvest.2014.100

146. Fukai T, Ushio-Fukai M, Kaplan JH (2018) Copper transporters and copper chaperones: roles in cardiovascular physiology and disease. Am J Physiol-Cell Physiol 315:C186–C201. https://doi.org/10.1152/ajpcell.00132.2018

147. Schuschke DA, Saari JT, Miller FN (2001) Leukocyte–endothelial adhesion is impaired in the cremaster muscle microcirculation of the copper-deficient rat. Immunol Lett 76:139–144. https://doi.org/10.1016/S0165-2478(01)00171-7

148. Kumar V, Kalita J, Bora HK, Misra UK (2016) Relationship of antioxidant and oxidative stress markers in different organs following copper toxicity in a rat model. Toxicol Appl Pharmacol 293:37–43. https://doi.org/10.1016/j.taap.2016.01.007

149. Kishimoto T, Fukuzawa Y, Abe M, et al (1992) Injury to Cultured Human Vascular Endothelial Cells by Copper (CuSO4). Nippon Eiseigaku Zasshi 47:965–970. https://doi.org/10.1265/jjh.47.965

150. Hu GF (1998) Copper stimulates proliferation of human endothelial cells under culture. J Cell Biochem 69:326–335. https://doi.org/10.1002/(sici)1097-4644(19980601)69:3<326::aid-jcb10>3.0.co;2-a

151. Wang L, Chen Z, Li Y, et al (2019) Optical coherence tomography angiography for noninvasive evaluation of angiogenesis in a limb ischemia mouse model. Sci Rep 9:5980. https://doi.org/10.1038/s41598-019-42520-3

152. Chen M, Luo Y, Xu J, et al (2019) Copper Regulates the Susceptibility of Zebrafish Larvae to Inflammatory Stimuli by Controlling Neutrophil/Macrophage Survival. Front Immunol 10:2599. https://doi.org/10.3389/fimmu.2019.02599

153. Gaupp R, Ledala N, Somerville GA (2012) Staphylococcal response to oxidative stress. Front Cell Infect Microbiol 2:. https://doi.org/10.3389/fcimb.2012.00033

154. Wang K-C, Garmire LX, Young A, et al (2010) Role of microRNA-23b in flow-regulation of Rb phosphorylation and endothelial cell growth. Proc Natl Acad Sci 107:3234–3239. https://doi.org/10.1073/pnas.0914825107

155. Ou H, Xiao X, Jiang Y, et al (2018) Expression of microRNA-23b in patients with sepsis and its effect on leukocytes and the expression of E-selectin and ICAM-1. Exp Ther Med. https://doi.org/10.3892/etm.2018.6759

156. Wang L, Bammler TK, Beyer RP, Gallagher EP (2013) Copper-Induced Deregulation of microRNA Expression in the Zebrafish Olfactory System. Environ Sci Technol 47:7466–7474. https://doi.org/10.1021/es400615q

157. on behalf of the Infection, Inflammation, Immunology and Immunisation (I4) section of the ESPR, Molloy EJ, Wynn JL, et al (2020) Neonatal sepsis: need for consensus definition, collaboration and core outcomes. Pediatr Res 88:2–4. https://doi.org/10.1038/s41390-020-0850-5

Appendix

Appendix

Communications

5ème Forum médical international de Chlef (05/05/2018)

INFECTIONS NOSOCOMIALES NEONATAL ET LA RESISTANCE AUX ANTIBIOTIQUES

A.Fatmi[1], S. A. Rebiahi[2], N. Chabni[3], H. Zarrouki[2], Y. Elhabiri[2], S. BenMansour[4], C. E. Smahi[4]

3ème journée nationales médico-chirurgicales et formation médicale continue (19 et 20/10/2018)

INFECTIOUS MORTALITY AND MORBIDTY AT THE NEONATOLOGY SERVICE LEVEL OF THE MATERNAL-CHILD SPECIALIZED HOSPITAL IN TLEMCEN 2017

A.Fatmi[1], S. A. Rebiahi[2], N. Chabni[3], H. Zarrouki[2], Y. Elhabiri[2], S. BenMansour[4], C. E. Smahi[4]

4ème journée nationales médico-chirurgicales et formation médicale continue (18 et 19/10/2019)

LA PLACE DE SEPTICÉMIE NÉONATALE AU NIVEAU DU SERVICE DE NÉONATOLOGIE DE L'EHS MÈRE- ENFANT DE TLEMCEN PENDANT DEUX ANNÉES

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Molecular Medicine

SHORT REPORT Open Access

miRNA-23b as a biomarker of culture-positive neonatal sepsis

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Abstract

Background: Neonatal sepsis remains an important cause of morbidity and mortality. The ability to quickly and accurately diagnose neonatal sepsis based on clinical assessments and laboratory blood tests remains difficult, where haemoculture is the gold standard for detecting bacterial sepsis in blood culture. It is also very difficult to study because neonatal samples are lacking.

Methods: Forty-eight newborns suspected of sepsis admitted to the Neonatology Department of the Mother-Child Specialized Hospital of Tlemcen. From each newborn, a minimum of 1–2 ml of blood was drawn by standard sterile procedures for blood culture. The miRNA-23b level in haemoculture was evaluated by RT-qPCR.

Results: miR-23b levels increased in premature and full-term newborns in early onset sepsis ($p < 0.001$ and $p < 0.005$ respectively), but lowered in late onset sepsis in full-term neonates ($p < 0.05$) compared to the respective negative controls. miR-23b levels also increased in late sepsis in the negative versus early sepsis negative controls ($p < 0.05$). miR-23b levels significantly lowered in the newborns who died from both sepsis types ($p < 0.0001$ and $p < 0.05$ respectively). In early sepsis, miR-23b and death strongly and negatively correlated (correlation coefficient = − 0.96, $p = 0.0019$). In late sepsis, miRNA-23b and number of survivors (correlation coefficient = 0.70, $p = 0.506$) positively correlated.

Conclusions: Lowering miR-23b levels is an important factor that favours sepsis development, which would confirm their vital protective role, and strongly suggest that they act as a good marker in molecular diagnosis and patient monitoring.

Keywords: Early-onset sepsis, Haemoculture, Late-onset sepsis, miR-23b, Newborns

Introduction

During the neonatal period, the immune system is still immature, and most immune responses are ensured by innate immunity, triggered following intimate contact between immune cells and microbes. In newborns, altered microbiota or microbial deprivation, as well as reduced microbial diversity, greatly increase the risk of immune dysregulation and proneness to inflammatory diseases. This makes neonates very fragile and more sensitive to several infectious diseases (Kumar and Bhat 2016; Lucignano et al. 2011).

Nowadays, neonatal sepsis is one of the most dangerous conditions to affect newborns during the first 28 days of life, and is a first-order public health system problem given its very high risk of mortality and morbidity (Bhandari 2014; Panwar et al. 2017). It is frequently divided into two types according to onset time: early-onset sepsis (EOS), when the process develops during the first 72 h of life; late-onset sepsis (LOS), when it

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occurs after the first 72 h. Unlike to fungi and parasites, bacteria and viruses are the commonest causative agents involved in neonatal sepsis aetiology (Ansari et al. 2015; Cortese et al. 2016).

Sepsis is commonly diagnosed by microbiological blood culture, but this can take days to perform, can suffer contamination and provide false-negative results. An empirical antibiotic therapy approach in neonatal sepsis is common clinical practice. In the presence of suspected bacterial infection, the use of random antibiotics is often unnecessary and prolongs the treatment of many uninfected newborns. Increasing the risk of multi-resistant strains emerging, however, or delaying or stopping antibiotic use in septicaemic newborns can also be catastrophic given rapid disease progression (Ng 2004; Shane et al. 2017). In addition, caring for newborns in specific hospital departments is a drain on human and financial resources (Atif et al. 2008; Wagstaff et al. 2019).

MicroRNAs (miRNAs), a class of small single-stranded non-coding regulatory RNAs of about 19 to 22 nucleotides, are involved in a wide range of biological processes and have opened a new window of hope to diagnose, and even treat, various diseases. miRNA binds to specific mRNA molecules to inhibit the expression of target genes or to degrade mRNA, which then contributes to cell proliferation, differentiation, development, metabolism, apoptosis and other physiological activities (Wu et al. 2015; Lenkala et al. 2014). Given their role in various cellular processes, recent studies reveal how miRNAs may have the potential to be an early biomarker in a number of diseases, including sepsis (Wang et al. 2010). Among microRNAs, we can identify miR-23b. Chromosomal region 9q22.32 produces miR-23b. The combined body of available works suggests that miR-23b expression is not only modulated by a diverse array of stimuli in cells from different lineages, but also participates in multiple gene regulatory feedback loops (Wang et al. 2018). Nevertheless, the role of miRNAs in neonatal sepsis has not been widely explored. It is noteworthy that miR-23b is a proven and important regulator of the innate immune response in both cancer and several inflammatory processes (Zhu et al. 2012). In addition, some studies show that microR-23b expression in the peripheral blood of sepsis patients is related to the manifestation of an inflammatory state and may, therefore, be used to evaluate the severity and prognosis of adults patients with this disease (Ou et al. 2018). In another study, miR-23b is proposed as an essential contributor to cardiac fibrosis activation to mediate the development of myocardial dysfunction in late sepsis. This report suggests that blocking miR-23b expression might be an effective approach to prevent sepsis-induced cardiac dysfunction (Zhang et al. 2018). Another study reveals that miR-23b inhibition down-regulates the expression of programmed death ligand-1 (PD-L1) on splenic T lymphocytes of septic mice. This discovery opens up new therapeutic pathways in late stages of the septic phase (Beltrán-García et al. 2020). Finally, another study demonstrates that miR-23b is an anti-inflammatory factor that negatively regulates the inflammatory responses induced by lipopolysaccharide (LPS) by targeting metalloproteinase 10 (ADAM10) (Zhang et al. 2019a).

Haemocultures are the "gold standard" for identifying bacterial and fungal infections in the bloodstream. However, they are limited by large volume requirements to maximise sensitivity and often imply long incubation times. To address some of these limitations, many advances have been developed to improve sensitivity and to reduce the time required to identify the cause of bloodstream infections. Molecular amplification techniques have been developed to replace the incubation step in blood culture targeting conserved regions of microbial genomes for amplification, such as rRNA genes and interspace region 16S–23S (Tsalik et al. 2010; Gurtler and Stanisich 1996; Draz et al. 2013).

In this study, we attempted to estimate the expression levels of candidate circulating miR-23b in small cohorts of newborns diagnosed with early (EOS) or late (LOS) sepsis by microbiological blood culture test in the Neonatology Department of Mother & Child Specialized Hospital Establishment of Tlemcen (northwest Algeria). We show for the first time that miR-23b can be considered a potential marker of sepsis in haemocultures from neonate peripheral blood samples. Hence, we demonstrated that miR-23b levels increased in EOS, but lowered in LOS, compared to the respective negative controls. These levels also increased in the LOS negative controls compared to the EOS negative controls. Therefore, the drop in miR-23b levels would undoubtedly be an important factor that favours sepsis development, which would confirm their vital protective role on the one hand, and would strongly suggest their use as a good marker in both molecular diagnosis and patient monitoring on the other hand.

Patients and methods

Ethical aspects

The present study was approved by the Local Ethics Committee of Tlemcen University. Parents or legal guardians gave written informed consent so that the samples from all the participating infants could be used according to the Declaration of Helsinki.

Study population

Of the 2561 newborns admitted during a 12-month period to the Neonatology Department of Mother &

Child Specialized Hospital Establishment (EHS, *Etablissement Hospitalier Spécialisé Mère-Enfant*) of Tlemcen (northwest Algeria), 254 (9.91%) newborns with sepsis were recorded. Forty-eight cases aged up to 28 days with clinical features of sepsis (e.g. fever, respiratory distress, bradycardia, tachycardia, convulsions, cyanosis), and an association, or not, with premature rupture of membranes (PROM), and abnormal amniotic liquid as risk factors (Singer et al. 2016), who met the neonatal sepsis inclusion criteria, were recruited in a prospective cohort study. The exclusion criteria included those patients without sepsis clinical features or those who had received antibiotherapy before sampling. Newborns included 20 females and 28 males. The 48 patients were randomly divided into two groups of 27 EOS, including nine cases of preterm newborns and 21 LOS patients.

Samples for blood haemoculture

Peripheral blood samples (1–2 mL) were inoculated into aerobic bottles containing paediatric haemoculture medium (BIOSCAN, Sétif, Algeria) to be incubated at 37 °C for 4–6 h with agitation. Aliquots of 2 mL were collected and stored at − 80 °C until RNA extraction.

Total RNA extraction, including miRNA, was performed with a minimum of 200 μL of cell-free supernatant obtained after centrifuging an aliquot at 1200 rpm for 10 min using the miRNeasy Serum/Plasma kit (Qiagen, Valencia, Spain) according to the manufacturer's protocol. RNA was eluted with 20 μL of RNase-free water and was then quantified in a NanoDrop ND 2000 UV spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Reverse transcription PCR and real-time qPCR

Total RNA (1 μL) was converted into complementary DNA (cDNA) by reverse transcriptase using the miRNA TaqMan reverse transcription kit and miRNA-specific stem and loop primers (Part No. 4366597, Applied Biosystems. Inc., CA, USA). Real-time PCR was performed in an Applied BioSystem 7900HT Thermocycler (Applied Biosystems/Thermo Fisher, USA) with 40 cycles. The primers herein used were designed for miRNA-23b (hsa-miR-23b (Assay ID 002126). Thermo Fisher, CA, USA), and U6 snRNA (U6-snRNA (Assay ID 001973). Thermo Fisher, CA, USA), was used for standardisation purposes by the delta-delta CT method ($2^{-\Delta\Delta CT}$).

Statistical analysis

The results represent the mean (± standard deviation) of the median values of three independent replicate experiments. Analyses of variance were carried out by Mann Whitney *U* or Kruskal–Wallis non-parametric tests using GraphPad Prism 8.0.1 (244) as data were not normally distributed (Olsen 2003). *P*-value < 0.05 was considered statistically significant.

Results and discussion

Forty-eight haemocultures were performed in this study, of which 18.75% were premature and 81.25% at-term. Gender, temperature, heart rate, respiratory rate, glycaemia and caesarean vs. vaginal delivery characteristics were not statistically different between the control and the positive haemoculture groups. However, neonates' weight in both sepsis types was significantly different to C-reactive protein (CRP), which significantly differed in the EOS neonates. The clinical information of the 48 patients is shown in Table 1.

Changes in the miRNA-23b expression levels in early onset sepsis

The miR-23b expression levels in the neonatal sepsis samples were analysed by the quantitative real-time PCR method. Our results showed that, compared to the control group, the miR-23b expression levels significantly differed in the neonatal sepsis samples either in the at-term or premature neonates ($p < 0.001$ KW) (Fig. 1). The miR-23b expression significantly lowered in the neonates who died of sepsis ($p < 0.0001$, $p < 0.05$ at-term and premature infants, respectively), and significantly increased in the neonates who survived with a positive haemoculture ($p < 0.005$, $p < 0.001$). These results reveal that miR-23b expression correlates with sepsis progression.

Changes in the miR-23b expression levels in late onset sepsis

Figure 2 shows how the miR-23b expression in LOS significantly lowers in both the dead and surviving newborns with a positive haemoculture, with $p < 0.005$ and $p < 0.05$, respectively, compared to the controls and for all comparisons ($p < 0.05$ KW). Two cases presenting the clinical signs of sepsis died, but the haemoculture was negative. In this case, we recorded a significant drop in the miR-23b level with a negative haemoculture ($p < 0.05$). This case was considered a false-negative haemoculture, probably due to the sampling time (Hall and Lyman 2006) or another limitation, like the presence of unculturable or fastidious microorganisms that could decrease its sensitivity (Jordana-Lluch et al. 2014).

Change in the miRNA-23b expression levels in newborns in two different stages

The differences between our results when comparing EOS and LOS led us to think back to the starting point before sepsis appeared. Figure 3 shows the miR-23b expression level after the first 72 h of life and beyond that time in the control patients. The results revealed a significant increase in the miR-23b level after 72 h of live

Table 1 Characteristics of the newborn patients with sepsis in the present study

EOS/LOS (n = 27, 21)	**Full-term patients**				**Premature patients**			***p***
	Co/NH (control) (9, 6)	**SP/PH (7, 12)**	**DP/PH (2, 1)**	**DP/NH (0, 2)**	**Co/NH (control) (4, 0)**	**SP/PH (2, 0)**	**DP/PH (3, 0)**	
Gender (M/F)								
EOS	5/4	4/3	1/1	–	1/3	1/1	2/1	NS
LOS	6/0	6/6	0/1	2/0	–	–	–	NS
Weight (kg)								
EOS	2.87 ± 0.76	3.16 ± 0.65	3.30 ± 0.14	–	2.01 ± 0.09	1.43 ± 0.23	1.63 ± 0.57	< 0.001
LOS	3.17 ± 0.86	3 ± 0.86	2.5 ± 0	2.9 ± 0.28	–	–	–	< 0.001
T (° C)								
EOS	36.17 ± 1.43	35.05 ± 1.42	34.5 ± 1.27	–	36.35 ± 2.30	33.07 ± 2.21	36.17 ± 1.20	NS
LOS	37.33 ± 2.85	38.3 ± 1.53	37.4 ± 0	39.05 ± 0.95	–	–	–	NS
HR (BPM)				–				
EOS	136 ± 16.37	143.6 ± 17.87	125 ± 9.9	–	120.5 ± 19.58	125 ± 7.07	150 ± 10	NS
LOS	151.8 ± 27	140.3 ± 17.27	180 ± 0	135 ± 7.07	–	–	–	NS
RR (BrPM)				–				
EOS	55.3 ± 14.56	55.7 ± 17.1	38 ± 5.66		58 ± 5.42	42 ± 2.83	62.67 ± 4.72	NS
LOS	50.7 ± 14.01	52.5 ± 6.1	52 ± 0	42 ± 2.83	–	–	–	NS
Gly (mg/dL)								
EOS	0.95 ± 0.48	0.63 ± 0.23	0.52 ± 0.60		0.73 ± 0.25	–	0.45 ± 0.15	NS
LOS	1.12 ± 0.22	0.65 ± 0.21	–	0.66 ± 0.17	–	–	–	NS
CRP (mg/dL)								
EOS	25.78 ± 17.87	41.17 ± 30.75	–	–	63 ± 88.25	–	–	< 0.0001
LOS	47 ± 81.37	39.87 ± 29.78	–	42 ± 25.45	–	–	–	NS
VD vs. CD				–			–	
EOS	5/4	5/2	1/1	–	2/2	1/1	1/2	NS
LOS	5/1	1/1	1/0	2/0	–	–	–	NS

Data are presented as the mean ± standard deviation (X ± SD)
BPM beats per minute, *BrPM* breaths per minute, *CF* Cardiac frequency, *Co/ NH* control newborns with negative haemoculture, *CRP* C-reactive protein, *DP/NH* patients who died with negative haemoculture, *DP/PH* patients who died with positive haemoculture, *EOS* early onset sepsis, *F* female, *Gly* glycaemia, *HR* Heart rate, *EOS* early onset sepsis, *LOS* late onset sepsis, *M* male, *NS* not significant, *RR* Respiratory rate, *SP/PH* patients who survived with positive haemoculture, *VD vs. CD* vaginal vs. caesarean delivery

performance $p < 0.05$ compared to that before the first 72 h of life.

The correlation between miR-23b, sepsis and death during the neonatal period

Figure 4 shows a very strong negative correlation between miR-23b and death in early sepsis patients (r = – 0.96, p = 0.002), the same for premature, with a negative correlation with miR-23b and death sepsis (r = – 0.89, p = 0.0001), but the miR-23b level correlated negatively with sepsis (r = – 0.81, p = 0.39). With late sepsis, a low negative non-significant correlation is observed between miR-23b and the appearance of sepsis (r = – 0.26, p = 0.32). Nevertheless, we show a positive correlation between miRNA-23b levels in both the control and non-survivor patients (r = 0.70, p = 0.506).

Infant and late foetal deaths are key factors when assessing a country's level of social protection (Say et al. 2009; Gonzalez and Gilleskie 2017). In neonatal sepsis, the leading treatment is antibiotics, which target the infecting pathogen, but not the inflammatory process that continues to increase. Therefore, an ideal treatment approach should include antimicrobial and anti-inflammatory drugs to neutralise the rising inflammatory cascade and the resulting "cytokine storm" in neonatal sepsis (Nedeva et al. 2019). Newborns immune status during the perinatal period differs from that of adults. Neonatal immune responses are generally directed against the generation of T helper type 1 (Th1)-related proinflammatory immune responses, while favouring the Th2-related anti-inflammatory /immunosuppressive response (Kollmann et al. 2012). This process represents an efficient strategy to address the unique challenges of

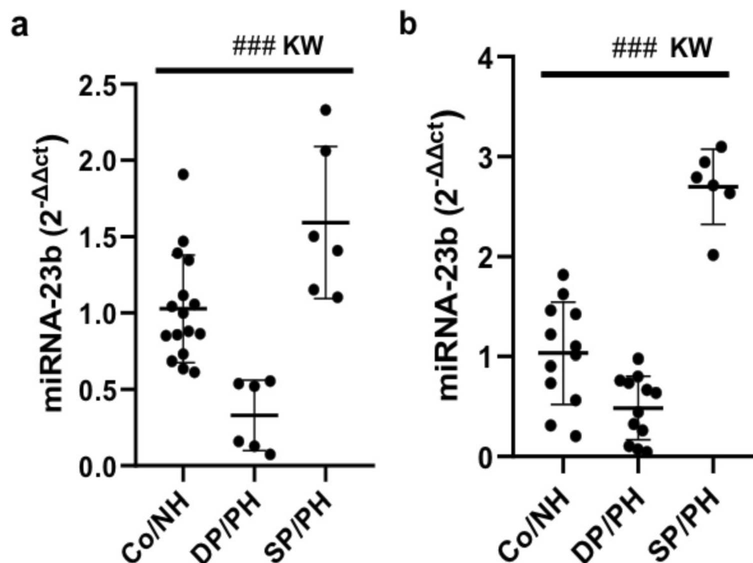

Fig. 1 Changes in the miRNA-23b expression levels in early onset sepsis. Scatter plot values a on the left represent the miR-23b level for EOS in the at-term newborns, measured by ($2^{-\Delta\Delta CT}$). While that scatter plot in b on the right represent the miR-23b level in the premature newborns. The line inside the boxes corresponds to the median values. For the at-term patients, Co/NH ($n = 9$), DP/PH ($n = 2$), SP/PH ($n = 7$). For the premature patients, Co/NH ($n = 4$), DP/PH ($n = 3$), SP/PH ($n = 2$). Co/NH: negative haemoculture, DP/PH: positive haemoculture in dead newborns, SP/PH: positive haemoculture in the newborns who survived. KW: Kruskal-Wallis. The sharp indicate significant differences highlighted between all groups using the Kruskal-Wallis test: ### $p < 0.001$

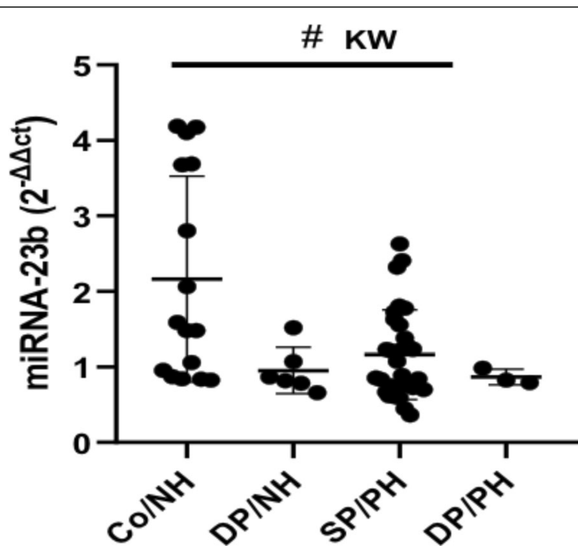

Fig. 2 Changes in the miR-23b expression levels in late onset sepsis. Scatter plot values represent the miR-23b level for LOS in at-term newborns, measured by ($2^{-\Delta\Delta CT}$). The line inside the boxes corresponds to the median values. Co/NH ($n = 6$), DP/NH ($n = 2$), SP/PH ($n = 12$), DP/PH ($n = 1$). Co/NH: negative haemoculture, DP/NH: negative haemoculture in the dead newborns, DP/PH: positive haemoculture in the newborns who died, SP/PH: positive haemoculture in the newborns who survived, KW: Kruskal-Wallis. The sharp indicate significant differences highlighted between all groups using the Kruskal-Wallis test: # $p < 0.05$

the neonatal period, including maintaining tolerance to maternal antigens in utero and balancing the transition from the sterile intrauterine environment to the antigen-rich outside world (Kollmann et al. 2012).

miRNAs are endogenous, non-coding, single-stranded RNAs (~ 22 nucleotides long) with the ability to degrade mRNA or inhibit translation, which then regulates gene expression at the post-transcriptional level (Wu et al. 2015). We know that the expression of ≥30% of human genes is controlled by miRNAs (Bartel 2004). miRNAs also regulate molecular signalling pathways and immune activities (Yu et al. 2018). The invasion of pathogenic microorganisms, followed by rapid miRNAs production, promote the release of inflammatory factors that cause immune hyperactivity, and induce apoptosis or degrading inflammatory factors that can provoke immunosuppression (Li et al. 2014; Chen et al. 2013).

The biomarkers frequently used in neonatal sepsis are still not completely conclusive. But have shown some potential for in vitro diagnoses (Kingsley Manoj Kumar and Vishnu Bhat 2015). Since their discovery, circulating miRNAs in human peripheral serum are used as biomarkers of various cancer types. The use of miRNAs as diagnostic and prognostic markers has extended to other diseases, including sepsis, but their role in infectious diseases has rarely been studied (Wang et al. 2013; Wang et al. 2012). One of the main obstacles to establish a well-defined link between miRNAs and sepsis lies in the fact that sepsis can be caused by very different factors

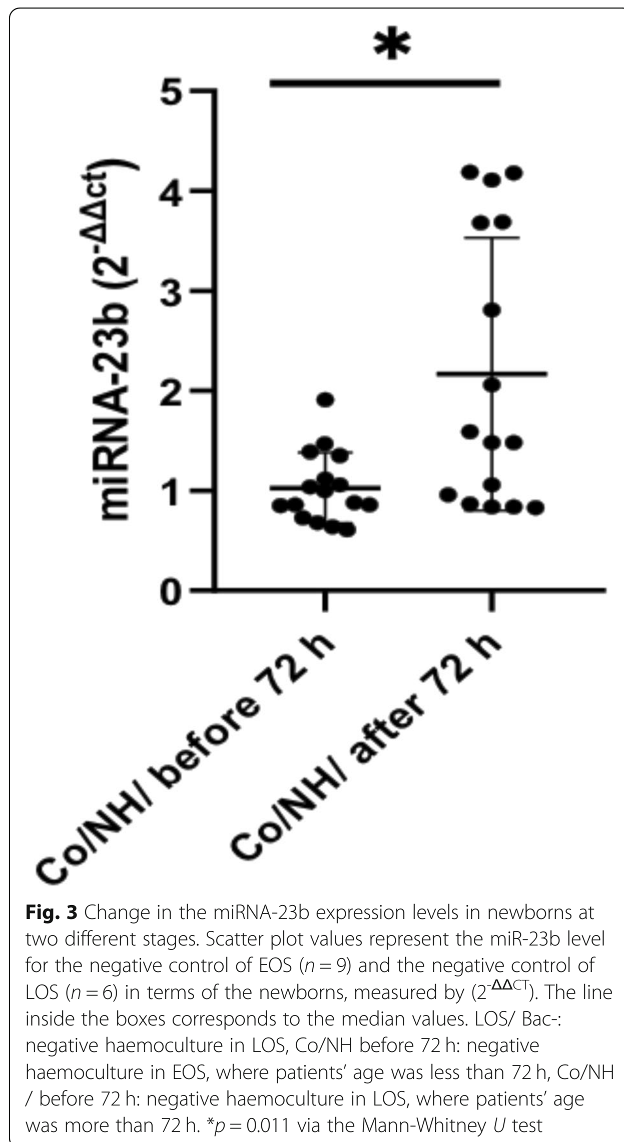

Fig. 3 Change in the miRNA-23b expression levels in newborns at two different stages. Scatter plot values represent the miR-23b level for the negative control of EOS ($n = 9$) and the negative control of LOS ($n = 6$) in terms of the newborns, measured by ($2^{-\Delta\Delta CT}$). The line inside the boxes corresponds to the median values. LOS/ Bac-: negative haemoculture in LOS, Co/NH before 72 h: negative haemoculture in EOS, where patients' age was less than 72 h, Co/NH / before 72 h: negative haemoculture in LOS, where patients' age was more than 72 h. *$p = 0.011$ via the Mann-Whitney *U* test

that cause similarities and differences, which influence the patient's situation itself and make sepsis so very complicated (Stearns-Kurosawa et al. 2011). This is why the association of miRNAs with sepsis diagnosis remains controversial (Zhang et al. 2019b).

miRNAs from different biological fluids can be used for the early prediction and evaluation of neonatal sepsis, where various miRNAs are down-regulated, and contribute to the initiation of the immune response to infection (Chen et al. 2014). To date, no studies are available on miRNAs in both neonatal sepsis types, i.e., EOS and LOS. To the best of our knowledge, no study has investigated miRNAs expression levels in haemocultures from septic patients and their change according to neonatal sepsis types.

The neonatal immune response to sepsis depends on the timing of onset, relative pathogens and developmental age (Sweeney et al. 2018), and is markedly different from the immune response in adults because of specific neonatal microbial susceptibility and atopic properties. Differences have been reported in the regulation of target gene expression by miRNAs in innate immunity (Yu et al. 2018). A study of ten immune-regulating miRNAs, whose expression significantly altered more than 2-fold in neonates with sepsis compared to uninfected neonates, showed that miRNA expression levels were altered, and that this alteration in miRNAs modulated the immune response during neonatal sepsis so as to represses inflammatory response (Chen et al. 2014). In another study (Cheng et al. 2018), low miRNA-26a levels have been correlated with the up-regulation of IL-6 expression in blood mononuclear cells and serum. Nevertheless, neither newborns age nor sepsis type has been specified. There are also reports indicating that miR-15a/16 can be used as a potential biomarker for the diagnosis and prognosis of neonatal sepsis, and that miRNA15a/16 regulation may limit the inflammatory response to LPS (Wang et al. 2015).

Although the discovery of miR-23b is recent (Wu et al. 2015; Ou et al. 2018), intense research efforts have been made to show that it is involved in various physiological and pathophysiological processes (Wang et al. 2018). So, it has been revealed as an essential moderator of several physiological pathways that regulate the differentiation of many cell lines, such as keratinocytes, chondrocytes and skeletal muscle. miR-23b also regulates inflammatory response in several autoimmune diseases through suppressing proinflammatory signalling pathways in resident cells, such as human fibroblast-likesynoviocytes, and in primary kidney cells and astrocytes from mice (Bordon 2012). miR-23b also plays a critical role in certain pathologies, including acute myocardial infarction, inflammatory heart diseases and sepsis-induced cardiac dysfunction (Grossi et al. 2018; Zhao et al. 2016), diabetic nephropathy (Zhao et al. 2016) and prostate cancer (Pimenta et al. 2018). We herein demonstrate for the first time the presence of miR-23b in haemocultures from neonatal sepsis and their interest for diagnosis and prognosis in early and late sepsis.

In sepsis, miR-23b has been reported to be down-regulated in peripheral blood mononuclear cells (PBMCs) from adult patients and in the LPS-induced THP-1 human monocytic cell line, and has been negatively correlated with the production of proinflammatory cytokines. Increased miR-23b expression has been shown to induce the down-regulation of proinflammatory cytokines production and LPS-stimulated apoptosis (Zhang et al. 2019a).

In the present study, we revealed that at-term birth, the neonates with negative haemocultures (the control

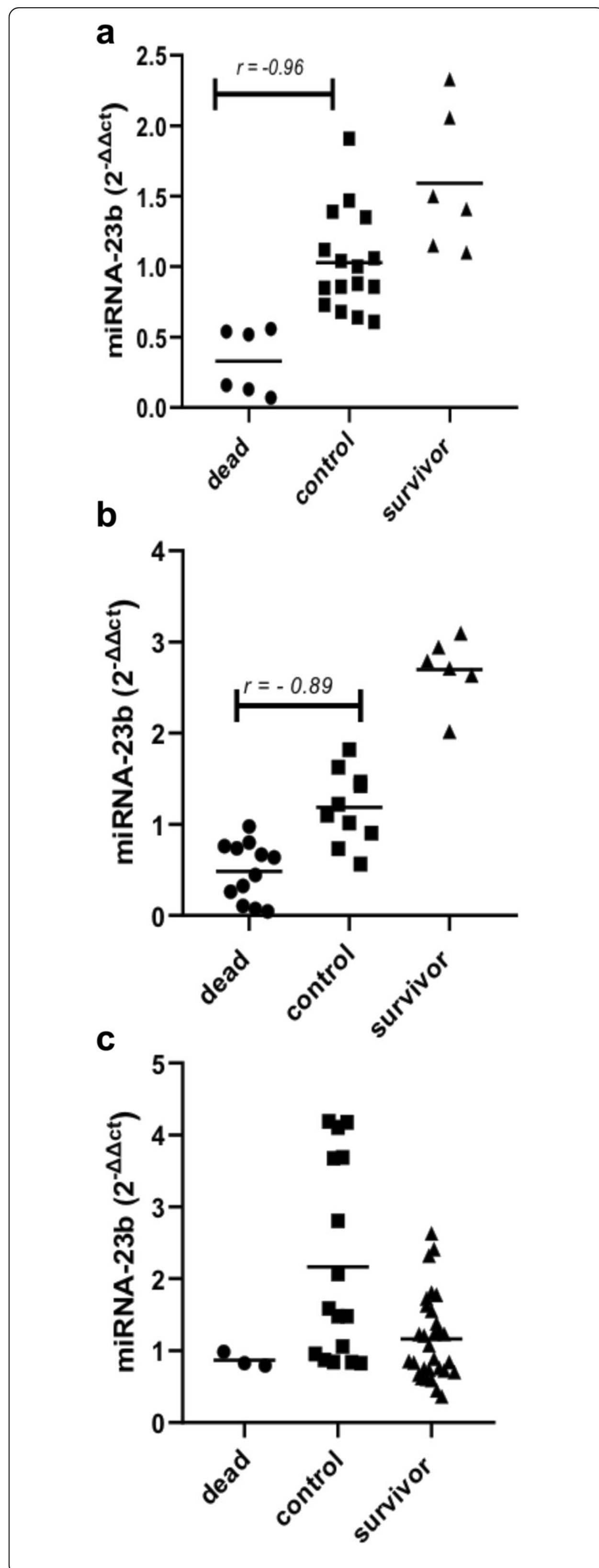

Fig. 4 Correlation between miRNA-23b expression and dead newborns with sepsis. Individual values represent the correlation with the miR-23b level, survivor and dead in newborns with sepsis. **a**: in early onset sepsis in at-term newborns, **b**: early onset sepsis in premature newborns, **c**: late onset sepsis in at-term newborns. Control: patients with negative haemoculture, dead: dead patients with sepsis, survivor: survivor patients with sepsis, *r*: correlation coefficient

group) presented low miR-23b levels during the first 72 h of life, which started to increase after 72 h. Conversely, the neonates with a positive haemoculture who did not survive infection always showed the lowest miR-23b levels, regardless of whether they were premature or born at term. In addition, the neonates with a positive haemoculture who survived infection had high miR-23b levels during the first 72 h of life, irrespectively of whether they were born at term or premature. Thus the increase in miR-23b levels during the first 72 h of life in septic neonates may be a potent prognostic factor for survival and a sensitive clinical marker in both preterm and at-term neonates.

It has been recently shown with an animal model of sepsis that miRNA profiles in $CD8^+$ T cells from adult and neonatal mice were surprisingly similar during infection, but infection miRNA levels differed when it was absent. In particular, marked differences were observed in the miR-29 and miR-130 expression levels between adult and neonatal cells before infection. Likewise, changes in the expression of messenger RNA targets have been noted for both miR-29 and miR-130 (Wissink et al. 2015).

Our study indicated a difference in the miR-23b expression levels in both EOS and LOS. The miR-23b expression levels increased in the EOS patients with a positive haemoculture and lowered in the LOS patients with either premature or full-term newborns. Nevertheless, we also observed a difference in expression over time in the control group before and after 72 h of birth. This could be due to differences in the genome expression patterns in newborns between EOS and LOS. Exclusively to newborns, uninfected status and host response to sepsis are significantly affected by time of birth (Wynn et al. 2015; Raymond et al. 2017), in which immune system development is a continuous process throughout embryogenesis and into childhood. Hence the different miRNAs expression in neonatal sepsis could be considered a developmental characteristic of the immune response (Chen et al. 2014). Early and late sepsis responses considerably differ depending on the postnatal age at the time of sepsis (Ng et al. 2018). By controlling postnatal age in studies on epigenetic changes during neonatal sepsis, we were able to better understand the immune mechanism in newborns and to

identify therapeutic targets. From these results, we suggest the possibility of using miR-23b levels as an in vitro diagnosis marker, which can be used to differentiate between EOS and LOS. miR-23b levels are up-regulated during the first 72 h of life and down-regulated over time during this period.

Conclusions

In this first report, we demonstrate the usefulness of miRNAs in haemocultures from neonates, and the role of miR-23b as a potent biomarker in sepsis. This study could be of much interest in not only research, but also in Translational Medicine, and more specifically in Neonatal Infectiology, where the use of large volumes of blood is not possible. *In fine*, this study provides additional elements into the molecular approach for diagnosing and treating neonatal sepsis. These elements include three essential points: (i) miR-23b plays a vital role in neonatal sepsis; (ii) the expression of miR-23b differs during the neonatal period; (iii) miR-23b expression levels are up-regulated in EOS and down-regulated in LOS.

Abbreviations

BPM: Beats per minute; BrPM: Breaths per minute; CF: Cardiac frequency; Co/NH: Control newborns with negative haemoculture; CRP: C-reactive protein; DP/NH: Patients who died with negative haemoculture; DP/PH: Patients who died with positive haemoculture; EOS: Early-onset sepsis; F: Female; Gly: Glycaemia; HR: Heart rate; KW: Kruskal-Wallis; LOS: Late-onset sepsis; LPS: Lipopolysaccharide; M: Male; miRNAs: MicroRNAs; NH: Negative haemoculture; NS: Not significant; PBMCs: Peripheral blood mononuclear cells; PROM: Premature rupture of membranes; PD-L1: Programmed death ligand-1; RR: Respiratory rate; SP/PH: Patients who survived with positive haemoculture; VD vs. CD: Vaginal vs. caesarean delivery

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Authors' contributions

AF: performed experiments, statistical analyses and drafted the manuscript. SAR: provided advice and participated in refreshing references. HZ: participated in microbial identification. HZ and YE: performed experiments.NC: financial support, study design and advice.SB: recruited eligible patients, traceability of samples and consents. JSIC performed experiments, JLGG: study design and interpretation of the results. MA: coordinated the study, interpretation of the results and proofread the manuscript. FVP: study design, interpretation of the results, financial support and proofread the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

No applicable.

Ethics approval and consent to participate

The present study was approved by the Local Ethics Committee of Tlemcen University. Parents or legal guardians gave written informed consent so that the samples from all the participating infants could be used according to the Declaration of Helsinki.

Consent for publication

All authors give the consent for publication.

Competing interests

The authors declare no conflict of interest.

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References

Ansari S, Nepal HP, Gautam R, et al. Neonatal septicemia in Nepal: early-onset versus late-onset. Int J Pediatr. 2015;2015:1–6. https://doi.org/10.1155/2015/379806.

Atif ML, Sadaoui F, Bezzaoucha A, et al. Prolongation of hospital stay and additional costs due to nosocomial bloodstream infection in an Algerian neonatal care unit. Infect Control Hosp Epidemiol. 2008;29:1066–70. https://doi.org/10.1086/591858.

Bartel DP. MicroRNAs. Cell. 2004;116:281–97. https://doi.org/10.1016/S0092-8674(04)00045-5.

Beltrán-García J, Osca-Verdegal R, Romá-Mateo C, et al. Epigenetic biomarkers for human sepsis and septic shock: insights from immunosuppression. Epigenomics. 2020. https://doi.org/10.2217/epi-2019-0329.

Bhandari V. Effective biomarkers for diagnosis of neonatal sepsis. J Pediatr Infect Dis Soc. 2014;3:234–45. https://doi.org/10.1093/jpids/piu063.

Bordon Y. MicroRNA-23b keeps TABs on tissue inflammation. Nat Rev Immunol. 2012;12:475. https://doi.org/10.1038/nri3250.

Chen C-Z, Schaffert S, Fragoso R, Loh C. Regulation of immune responses and tolerance: the microRNA perspective. Immunol Rev. 2013;253:112–28. https://doi.org/10.1111/imr.12060.

Chen J, Jiang S, Cao Y, Yang Y. Altered miRNAs expression profiles and modulation of immune response genes and proteins during neonatal sepsis. J Clin Immunol. 2014;34:340–8. https://doi.org/10.1007/s10875-014-0004-9.

Cheng Q, Tang L, Wang Y. Regulatory role of miRNA-26a in neonatal sepsis. Exp Ther Med. 2018. https://doi.org/10.3892/etm.2018.6779.

Cortese F, Scicchitano P, Gesualdo M, et al. Early and late infections in newborns: where do we stand? A review. Pediatr Neonatol. 2016;57:265–73. https://doi.org/10.1016/j.pedneo.2015.09.007.

Draz NI, Taha SE, Abou Shady NM, Abdel Ghany YS. Comparison of broad range 16S rDNA PCR to conventional blood culture for diagnosis of sepsis in the newborn. Egypt J Med Hum Genet. 2013;14:403–11. https://doi.org/10.1016/j.ejmhg.2013.05.004.

Gonzalez RM, Gilleskie D. Infant mortality rate as a measure of a country's health: a robust method to improve reliability and comparability. Demography. 2017;54:701–20. https://doi.org/10.1007/s13524-017-0553-7.

Grossi I, Salvi A, Baiocchi G, et al. Functional role of microRNA-23b-3p in cancer biology. MicroRNA. 2018;7:156–66. https://doi.org/10.2174/2211536607666180629155025.

Gurtler V, Stanisich VA. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology. 1996;142:3–16. https://doi.org/10.1099/13500872-142-1-3.

Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19:788–802. https://doi.org/10.1128/CMR.00062-05.

Jordana-Lluch E, Giménez M, Quesada MD, et al. Improving the diagnosis of bloodstream infections: PCR coupled with mass spectrometry. Biomed Res Int. 2014;2014:1–8. https://doi.org/10.1155/2014/501214.

Kingsley Manoj Kumar S, Vishnu Bhat B. Current challenges and future perspectives in neonatal sepsis. Pediatr Infect Dis. 2015;7:41–6. https://doi.org/10.1016/j.pid.2015.10.005.

Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by toll-like receptors: distinct responses in newborns and the elderly. Immunity. 2012;37:771–83. https://doi.org/10.1016/j.immuni.2012.10.014.
Kumar SKM, Bhat BV. Distinct mechanisms of the newborn innate immunity. Immunol Lett. 2016;173:42–54. https://doi.org/10.1016/j.imlet.2016.03.009.
Lenkala D, LaCroix B, Gamazon ER, et al. The impact of microRNA expression on cellular proliferation. Hum Genet. 2014;133:931–8. https://doi.org/10.1007/s00439-014-1434-4.
Li YC, Chen Y, Liu W, Thadhani R. MicroRNA-mediated mechanism of vitamin D regulation of innate immune response. J Steroid Biochem Mol Biol. 2014;144:81–6. https://doi.org/10.1016/j.jsbmb.2013.09.014.
Lucignano B, Ranno S, Liesenfeld O, et al. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. J Clin Microbiol. 2011;49:2252–8. https://doi.org/10.1128/JCM.02460-10.
Nedeva C, Menassa J, Puthalakath H. Sepsis: inflammation is a necessary evil. Front Cell Dev Biol. 2019;7:108. https://doi.org/10.3389/fcell.2019.00108.
Ng PC. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed. 2004;89:F229–35.
Ng S, Strunk T, Jiang P, et al. Precision medicine for neonatal sepsis. Front Mol Biosci. 2018;5:70. https://doi.org/10.3389/fmolb.2018.00070.
Olsen CH. Review of the use of statistics in infection and immunity. Infect Immun. 2003;71:6689–92. https://doi.org/10.1128/iai.71.12.6689-6692.2003.
Ou H, Xiao X, Jiang Y, et al. Expression of microRNA-23b in patients with sepsis and its effect on leukocytes and the expression of E-selectin and ICAM-1. Exp Ther Med. 2018. https://doi.org/10.3892/etm.2018.6759.
Panwar C, Kaushik S, Kaushik R, Sood A. Correlation of neonatal and maternal clinico-hematological parameters as predictors of early onset neonatal sepsis. Int J Contemp Pediatr. 2017. https://doi.org/10.18203/2349-3291.ijcp20164516.
Pimenta RC, Viana NI, Amaral GQ, et al. MicroRNA-23b and microRNA-27b plus flutamide treatment enhances apoptosis rate and decreases CCNG1 expression in a castration-resistant prostate cancer cell line. Tumor Biol. 2018;40:101042831880301. https://doi.org/10.1177/1010428318803011.
Raymond SL, Stortz JA, Mira JC, et al. Immunological defects in neonatal sepsis and potential therapeutic approaches. Front Pediatr. 2017;5:14. https://doi.org/10.3389/fped.2017.00014.
Say L, Souza JP, Pattinson RC. Maternal near miss – towards a standard tool for monitoring quality of maternal health care. Best Pract Res Clin Obstet Gynaecol. 2009;23:287–96. https://doi.org/10.1016/j.bpobgyn.2009.01.007.
Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet. 2017;390:1770–80. https://doi.org/10.1016/S0140-6736(17)31002-4.
Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315:801. https://doi.org/10.1001/jama.2016.0287.
Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, et al. The pathogenesis of sepsis. Annu Rev Pathol Mech Dis. 2011;6:19–48. https://doi.org/10.1146/annurev-pathol-011110-130327.
Sweeney TE, Wynn JL, Cernada M, et al. Validation of the sepsis MetaScore for diagnosis of neonatal sepsis. J Pediatr Infect Dis Soc. 2018;7:129–35. https://doi.org/10.1093/jpids/pix021.
Tsalik EL, Jones D, Nicholson B, et al. Multiplex PCR to diagnose bloodstream infections in patients admitted from the emergency department with sepsis. J Clin Microbiol. 2010;48:26–33. https://doi.org/10.1128/JCM.01447-09.
Wagstaff JS, Durrant RJ, Newman MG, et al. Antibiotic treatment of suspected and confirmed neonatal sepsis within 28 days of birth: a retrospective analysis. Front Pharmacol. 2019;10:1191. https://doi.org/10.3389/fphar.2019.01191.
Wang H, Zhang P, Chen W, et al. Four serum microRNAs identified as diagnostic biomarkers of sepsis. J Trauma Acute Care Surg. 2012;73:850–4. https://doi.org/10.1097/TA.0b013e31825a7560.
Wang H, Zhang P, Chen W, et al. Characterization and identification of novel serum MicroRNAs in sepsis patients with different outcomes. Shock. 2013;39:480–7. https://doi.org/10.1097/SHK.0b013e3182940cb8.
Wang J, Yu M, Yu G, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun. 2010;394:184–8. https://doi.org/10.1016/j.bbrc.2010.02.145.
Wang W, Wang Y, Liu W, van Wijnen AJ. Regulation and biological roles of the multifaceted miRNA-23b (MIR23B). Gene. 2018;642:103–9. https://doi.org/10.1016/j.gene.2017.10.085.
Wang X, Wang X, Liu X, et al. miR-15a/16 are upreuglated in the serum of neonatal sepsis patients and inhibit the LPS-induced inflammatory pathway. Int J Clin Exp Med. 2015;8:5683–90.
Wissink EM, Smith NL, Spektor R, et al. MicroRNAs and their targets are differentially regulated in adult and neonatal mouse CD8+ T cells. Genetics. 2015;201:1017–30. https://doi.org/10.1534/genetics.115.179176.
Wu M, Gu J-T, Yi B, et al. microRNA-23b regulates the expression of inflammatory factors in vascular endothelial cells during sepsis. Exp Ther Med. 2015;9:1125–32. https://doi.org/10.3892/etm.2015.2224.
Wynn JL, Guthrie SO, Wong HR, et al. Postnatal age is a critical determinant of the neonatal host response to sepsis. Mol Med. 2015;21:496–504. https://doi.org/10.2119/molmed.2015.00064.
Yu H-R, Huang L-H, Li S-C. Roles of microRNA in the immature immune system of neonates. Cancer Lett. 2018;433:99–106. https://doi.org/10.1016/j.canlet.2018.06.014.
Zhang H, Caudle Y, Shaikh A, et al. Inhibition of microRNA-23b prevents polymicrobial sepsis-induced cardiac dysfunction by modulating TGIF1 and PTEN. Biomed Pharmacother. 2018;103:869–78. https://doi.org/10.1016/j.biopha.2018.04.092.
Zhang W, Jia J, Liu Z, et al. Circulating microRNAs as biomarkers for sepsis secondary to pneumonia diagnosed via sepsis 3.0. BMC Pulm Med. 2019b;19:93. https://doi.org/10.1186/s12890-019-0836-4.
Zhang W, Lu F, Xie Y, et al. miR-23b negatively regulates sepsis-induced inflammatory responses by targeting ADAM10 in human THP-1 monocytes. Mediat Inflamm. 2019a;2019:1–13. https://doi.org/10.1155/2019/5306541.
Zhao B, Li H, Liu J, et al. MicroRNA-23b targets Ras GTPase-activating protein SH3 domain-binding protein 2 to alleviate fibrosis and albuminuria in diabetic nephropathy. J Am Soc Nephrol. 2016;27:2597–608. https://doi.org/10.1681/ASN.2015030300.
Zhu S, Pan W, Song X, et al. The microRNA miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TAB2, TAB3 and IKK-α. Nat Med. 2012;18:1077–86. https://doi.org/10.1038/nm.2815.

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Abstract

Background: Neonatal sepsis represent a major challenge in public health, where is the third most common cause of death of newborns. Neonatal sepsis characterized by misunderstood either at the molecular or at the cellular level. Here, in our study, we investigated the role of miRNA-23b in neonatal sepsis, and we studied the effect of reactive oxygen species in the activity of endothelial cells in sepsis.

Methods: Our study has two main parts. A prospective study based on Fifty-four newborns suspected sepsis. By RT-qPCR, we quantified the level of miRNA-23b in hemoculture. Via an *ex-vivo* model, we used the umbilical cord, which's divided into four groups. Where the exposure to oxidative stress caused by $CuSO_4$ and sepsis by *Staphylococcus aureus*. After 1 hour of incubation, we isolated endothelial cells and studied them.

Results: our study showing the relationship between sepsis and miRNA-23b, where We have shown that miRNA-23b levels increased in premature and full-term newborns in the case of EOS ($p < 0.001$ and $p < 0.005$ respectively), but decreased in LOS ($p < 0.005$). And proved the negative correlation between newborns who died from sepsis and miRNA-23b level. In the *ex-vivo* model, we have shown endothelial cells behaved differently against bacteria in the four conditions With betamethasone and without CuSO4, we noted the translocation of bacteria to the blood with a decrease in the level of miR-23b. Injection of CuSO4 into the blood induces a change in the activity of ECs and neutralizes the level of miR-23b, contribute to the defense against the translocation of bacteria in the blood.

Conclusions: The decrease in miRNA-23b levels would undoubtedly be an important factor favoring the development of neonatal sepsis. In addition to the protective role of copper-induced oxidative stress, activated endothelial cells promoting the pro-inflammatory response **in the blood may help prevent the translocation of S. aureus to the blood.**

Key words: Neonatal Sepsis, Endothelial Cells, Reactive Oxygen Species (ROS), miRNA-23b.

Résumé

Introduction : La septicémie néonatale représente un problème majeur de santé publique où elle est la troisième cause de décès chez les nouveau-nés. Septicémie néonatale caractérisée par une incompréhension que ce soit au niveau moléculaire ou cellulaire. Ici, dans notre étude, nous avons étudié le rôle du miARN-23b dans la septicémie néonatale, et nous avons étudié l'effet des espèces réactives de l'oxygène dans l'activité des cellules endothéliales dans la septicémie néonatale.

Matériel et Méthodes : Notre étude comporte deux parties principales. Une étude prospective basée sur Cinquante-quatre nouveau-nés suspectés de septicémie. Par RT-qPCR, nous avons quantifié le niveau de miRNA-23b en hémoculture. Via un modèle *ex-vivo*, nous avons utilisé le cordon ombilical, qui est divisé en quatre groupes. Où L'exposition au stress oxydatif causé par le $CuSO_4$ et la septicémie par *Staphylococcus aureus*. Après 1 heure d'incubation, nous avons isolé les cellules endothéliales et les avons étudiées.

Résultats : notre étude montrant la relation entre la septicémie et le miRNA-23b, où nous avons montré que les niveaux de miRNA-23b augmentaient chez les nouveau-nés prématurés et à terme dans le cas de septicémie précoce ($p < 0{,}001$ et $p < 0{,}005$ respectivement), mais a diminué en cas de septicémie tardif ($p < 0{,}005$). Et a prouvé la corrélation négative entre les nouveau-nés décédés par septicémie et le niveau de miRNA-23b. Dans le modèle *ex-vivo*, nous avons montré que les cellules endothéliales se comportaient différemment contre les bactéries dans les quatre conditions. Avec betaméthasone et sans $CuSO_4$, nous avons noté la translocation de bactéries au sang avec une diminution du taux de miR-23b. L'injection de $CuSO_4$ dans le sang induit une modification de l'activité des EC et neutralise le taux de miR-23b, contribue à la défense contre le Translocation de bactéries au sang.

Conclusions : La diminution des niveaux de miARN-23b serait sans aucun doute un facteur important favorisant le développement de la septicémie néonatale. En plus du rôle protecteur du stress oxydatif induit par le cuivre, les cellules endothéliales activées favorisant la réponse proinflammatoire dans le sang peuvent aider à prévenir la translocation de *S. aureus* au sang.

Mots clés : Septicémie Néonatale, Cellules Endothéliales, Espèces Réactives de l'Oxygène (ROS), miRNA-23b.

الملخص

المقدمة: يمثل الإنتان الوليدي مشكلة صحية عامة كبيرة حيث يعتبر السبب الرئيسي الثالث للوفاة عند الأطفال حديثي الولادة. يتميز الإنتان الوليدي بعدم الفهم سواء على المستوى الجزيئي أو الخلوي. هنا في دراستنا، قمنا بالتحقيق في دور ميرنا 23 ب في تعفن الدم الوليدي، ودرسنا تأثير أنواع الأكسجين التفاعلية على نشاط الخلايا البطانية في تعفن الدم حديثي الولادة.

لطريقة: دراستنا تتكون من جزأين رئيسيين. دراسة مستقبلية على أساس أربعة وخمسين مولودًا يشتبه في إصابتهم بالإنتان. بواسطة RT-qPCR، قمنا بتحديد مستوى ميرنا23ب في سائل زراعة الدم.باستخدام نموذج خارج الجسم الحي، استخدمنا الحبل السري، والذي يقسم إلى أربع مجموعات. حيث التعرض للإجهاد التأكسدي يتم بواسطة عن $CuSO_4$ والإنتان بواسطة *Staphylococcus aureus*. بعد ساعة واحدة من الحضانة، قمنا بعزل الخلايا البطانية ودرسناها.

النتائج: توضح دراستنا العلاقة بين تعفن الدم وميرنا 23ب، حيث أظهرنا أن مستويات ميرنا 23 ب زادت في الخدج والمواليد الناضجين الذين يعانون من الانتان الدم المبكر (P <0.001 و P <0.005 على التوالي)، ولكنها انخفضت في الإنتان المتأخر (P <0.005). وثبت الارتباط السلبي بين حديثي الولادة الذين ماتوا بسبب الإنتان ومستوى ميرنا 23 ب. في نموذج خارج الجسم الحي، أظهرنا أن الخلايا البطانية تتصرف بشكل مختلف ضد البكتيريا في جميع الظروف الأربعة. مع البيتاميثازون وبدون CuSO4، لاحظنا انتقال البكتيريا إلى الدم مع انخفاض مستوى miR-23b. يؤدي حقن CuSO4 في الدم إلى حدوث تغيير في نشاط ECs ومحافظة على مستوى miR-23b، والذي يساهم بدوره في الدفاع ضد انتقال البكتيريا إلى الدم.

الاستنتاجات: الانخفاض في مستويات ميرنا 23 ب بلا شك عامل مهم في تطور الإنتان الوليدي. بالإضافة إلى الدور الوقائي للإجهاد التأكسدي الناجم عن النحاس، فالخلايا البطانية المنشطة تعزز الاستجابة المؤيدة للالتهابات في الدم قد تساعد في منع انتقال المكورات العنقودية إلى الدم

الكلمات المفتاحية: الإنتان الوليدي، الخلايا البطانية، أنواع الأكسجين التفاعلية، ميرنا 23-ب