International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
SJIF Impact Factor: 5.142 Volume 3, Issue 3 – 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-3-10

The prognostic significance of neutrophil polymorph and band counts in under-five children with sepsis in Umth

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Abstract

Sepsis of various aetiologies often reflected in haematological changes during childhood, and significantly contributes to childhood mortality and morbidity. The objective of the study is to determine haemogram and neutrophil band count as a way of assessing the process of pathogenesis in under five children with sepsis in the University of Maiduguri Teaching Hospital. 50 paediatric ppatient with sepsis, diagnosed clinically and microbiologically were enlisted into the study prospectively and consecutively. Haematological investigations were carried out by standard methods. The results showed that majority of the patients (44%) had left shift neutrophilia. The 26 right shift neutrophils had neutrophil segmentation index anomaly of 86.6%. The male patient had PCV of 0.33 + 0.07 and WBC count of 9.9 + 3.7 as against 0.35 $\pm$0.05 and 9.6 $\pm$2.8 for females. Average defferential leucocytes count for the patient was neutrophil 88%, lymphocytes 15%, eosinophils 3% monocytes 1% and Basophils 1%.In this study, under five children with sepsis in the majority had left-shift neutrophilia suggesting acute infection. Those with right shift showed reactive neutrophil segmentation index anomaly.

Keywords: Sepsis, haematological changes, neutrophil band count, paediatric patient.

Introduction

Sepsis is a serious medical condition characterized by a whole- body inflammatory state called systemic inflammatory responds (SIRs) and the presence of known or suspected infection. The body may develop this inflammatory response to microbes in the blood and other body fluids, the term sepsis has a synonym septicaemia which is a term referring to the presence of pathogenic microorganisms in blood stream leading to sepsis (Dellinger et al., 2008).

Bacteraemia is the presence of bacteria in the blood stream whether associated with active disease or not. The transient bactereamia that follows surgical procedure such as dental manipulation as well as convalescent infection may have little significance in healthy individuals with functioning and intact immune system except children whose the integrity of their immune system is yet to be well defined and strong. Extensive bacteraemia when it is associated

with the release of bacterial toxins in to the systemic circulation can be fatal i.e. lead to bactereamic shock and eventually vascular collapse (Cheesbrough, 2005). Bactereamia usually follows the release of bacteria from sequestered sites such as urinary tract, gastro intestinal tract and less often respiratory tract in to the systemic circulation.

Septicemia is a condition of blood characterized by increasing high level of the bacteria in the circulatory system with resulting infections of tissues and organs. The symptoms and clinical manifestation of bactereamia are usually as a result of toxins (Exotoxins and Endotoxins) produce by the bacteria, the endotoxins are produced by gram negative bacteria such as group B streptococci, while the exotoxins are produced by gram positive bacteria such as *Klebsiella* species .It is this toxins that trigger off the immune system of the host.

Sepsis is a serious clinical condition in Sub Saharan Africa and Nigeria in particular especially the North-Eastern Nigeria. The record obtained from University of Maiduguri Teaching Hospital shows that children under five years of age are more susceptible and worst hit by sepsis. The record available shows that sepsis is a major clinical problem, probably due to ignorance, low educational background of all the congregating ethnic groups in the study and low disposal income, the problem is critical and very little or no work at all has been done to assess the significance of the neutrophil lobe account.

Sepsis has proven difficult over the years as there are no specific diagnoses for sepsis and even the prognosis for this infection is difficult to assess. The prevalence of sepsis among the children of less than five years and even neonates has been a great concerned over the years and so many works has been done on its classification, aetiology, diagnosis and treatment. A study carried out at strong memorial Hospital in Rochester. New York, 1994 shows that children between zero ages and five years meet virtually all the criteria for having sepsis (Army Strong Memorial Hospital Rochester. New York, 1994. Critical care med.)

Any kind of infection anywhere in the body can trigger sepsis. The infection may be caused by different types of germs, including bacteria, viruses e.tc.. The following factors may put a child at high risk of having sepsis;

i. Being malnourished (having poor nutrition) ii. Being born prematurely (before 37 weeks of gestation)

iii. Having a weak immune system

iv. Having heart defect, urinary tract problem, large burns or multiple injuries,

v. Taking medicines that decrease the body's ability to fight infection.

Sepsis is the systemic inflammatory response syndrome in children and clinically it is considered present wince;

The heart beat is greater than 90 minutes

The body temperature is greater than normal

The blood cell count is elevated (leucocytosis) >12,000

The respiratory rate is greater than normal >20/minute (Levy et al.,2003)

Other sign and symptoms of sepsis include vomiting, diarrhoea, bradycardia and abdorninal, tension. A lot of micro organisms have been implicated in its aetiology and among them are; *Escherichia coli, Klebsiella species. Streptococcus* spp.

In Africa generally, especially here in Nigeria there is paucity of records particularly on sepsis and its prognosis documented and very little or no research was carried out to check this. The clinical records available shows that under five children attending UMTH have the major problems of sepsis and the severity of the complications of sepsis are enormous, ranging from shock to multiple organ failure. Children with sepsis are required to be kept in intensive care unit (I.C.U.) with necessary regimen .These levels of care are only available in a few tertiary health institutions in Nigeria and only in one such place in the entire north east of Nigeria hence the need to examine sepsis as a major health threat among the children less than five years. The therapeutic management of sepsis requires virtually all health professionals and only laboratory results can be used to access the level of improvement or prognosis, the haemogram in particular is one of the most significant parameters needed as prognostic determiners (Martin et al.,2003).

Due to surrogate information error, many parents and guardians are not able to give the correct history of sepsis affecting then- children, this has necessitated the need for the study that will suggest whether die infection is recent or long standing based on neutrophil lobe count.

Objectives of the study

General objectives

The general objectives of the study is to investigate and determine the pattern of haemogram in children less than five years with sepsis

Specific objectives

The specific objectives include the following but are not limited to them;
To determine the packed cell volume and white blood cell counts
To determine the polymorph and bands counts
To detennine the differential leukocytes counts
To evaluate and interpret the result?

Subjects and method

Subjects

In this study, 50 under five years patients clinically and microbiologically diagnosed as having septicaemia were recruited into the study prospectively and consecutively. The patients were source; Pediiatric clinic and wards of the University of Maiduguri Teaching Hospital(UMTH). These patients who had sepsis secondary to Human immunodeftciency virus (HIV\ infection were excluded.

Study area

This study was carried out in university of Maiduguri Teaching Hospital (UMTH), a center of excellence laboratories haematology was used as the working center. University of Maiduguri teaching hospital is located in Borno state living within latitude 10°N and 14°N and longitude 110 30E and 14 0 45 E. Borao state which has an area of 61, 435sq Km is the largest state in the Nigeria in terms of land mass. Borno state is the capital of North eastern Nigeria, it shares borders with Adamawa to the south, Yobe to the west and Gombe to the south west. The state equally shares borders with countries such as Cameroon, Niger and Chad.

According to 2006 population census . Borno state has a population of above 4.1 million with density 60 inhabitant per square kilometer. The population is made up of diverse congregating ethnic groups from Nigeria and other part of Sub-Saharan Africa. But the dominant group are the Kanuri, Marghi, Bura, Gwoza, Shuwa and Fulani.

Methodology

Sample collection

Whole anti-coagulated blood (5ml) was collected from each recruited patient by vene-puncture into an EDTA bottle. The packed cell volume of each sample collected was immediately determined as well as the white blood cell count (WBC). Then the thin blood film was made from each sample.

Procedure for sample collection

Tourniquet was tied above the cubital area, the cubital area was palpated searching for anti-cubital vein,
when located it was clean with 75°o alcohol
to disinfect the area.
The disinfected area was allow to air dried
The syringe was set by pumping in and out to remove any free air in the
syringe.
The syringe was carefully inserted into the vein and blood was drawn gently
until the required volume was reached.
The tourniquet was released and the needle was drawn gently from the vein,
and dry cotton wool was placed on the punctured site.
The needle was
removed and the blood was dispensed into EDTA bottle.

Laboratory methods

All the laboratory procedures and methodologies were according to the description of Dacie et al.(2006).

Materials and reagents

The following materials and reagents were employed in carrying out this study but are not limited to them;
Slides
Blood (whole anti-coagulated blood)
Spreader
Cotton wool
Leishman stain
Syringe and needle
Counting chamber
Turk's solution
Capillary tube
Haematocrit centrifuge
Light microscope
Glass cover slips

Making of thin blood film

A thin blood film was made on each sample collected from each recruited subject.

Principle

Thin blood film is produced from an anti-coagulated blood on a slide smeared with a spreader to obtain less overlapping blood cells for microscopic examination.

Procedure

A drop of the anticoagulated whole blood on at the edge of a clean dust free slide
A spreader was positioned in front of the drop of blood, then push back to
touch it.
The spreader was then move up to three quarter of the slide with firmness and
consistency.
Each thin blood film appear more like a tongue.
The thin film made was allow to air dried and labelled.
It was then arranged for staining.

Staining of thin blood film

Leishman stain is one of the Romanowsky stain, the stain has a remarkable properties. Romanowsky dye make some distinctive staining properties and is dependent on two components i.e. Azure B (trimethyl thionin) and eosin Y (tetrabromopyescesin).

Principle

Leishman stain is a Romanowsky staining consisting of inethanol Azure B, and eosin Y. Methanol fixed the film, eosin Y stain the basic component. The differential uptake of the stain by the cell, is use to distinguish.

Procedure

The blood film was arranged on a staining rack
The thin blood film made was flooded with Leishman stain and allowed for 2 minutes.
It was then diluted with 7.8 buffer saline and allows staining for 8Minutes.
iv.The stained film was rinsed and the back wipes and cleans with cotton wool.

Packed cell volume (PCV)

The Haematocrit (packed cell volume) of each sample was determined using micro-haematocrit method.

Principle

Anti-coagulated blood is centrifuged in a sealed capillary tube at 11,000 rpm for 5 minutes, the volume of the packed red cell and percentage of the whole blood are determined by a special Haematocrit reader.

Procedure

The anti-coagulated whole blood was mixed gently
Then a heparinised micro-haematocrit tube was inserted deep and the blood
move into the tube by capillary (action) movement until it was % filled.
The second end of the tube was sealed with plastacine and placed on the
Haematocrit centrifuge
It was centrifuged at 11,00rpm for 5 minutes.
The level of the packed cell was determined using Haematocrit reader
PCV = packed RBC column height total blood column height.

White blood cell count (WBC)

White blood cell count of each recruited subject was performaing using turks solution and final results obtained from the 1st principle.

Principle

Whole blood is diluted 1 in 20 in acid reagent which haemolyses the red cells leaving white blood cells to be counted, the blood cells are counted microscopically using improved Neubauer counting chamber (haemocytometer) and the number of white blood cells (WBC) per litre of blood calculated.

Procedure

The freshly collected anti-coagulated blood was placed in a rotary mixer
while the dilution fluid was been prepared.
0.02ml (20µl) of the whole anti-coagulated blood was added to a 0.38ml
(380µl) of the Turk's solution in a Jin tubes.
It was mixed well and allow to stand for about 10 minutes to haemolyse the
red blood cells
The counting chamber was cleaned and cover slip was placed onto the
chamber
The diluted blood was tapped and using Pasteur pipettes was use to charge the chamber

The counting chamber was kept in a moist chamber for about 5 minutes to
allow the cells to settles down
The counting chamber was placed under microscope and the white cells
counted using X 10 objectives
The result of the counting from the four large squares was subjected to the
first principle
WBC $\frac{NXDF}{0.4}$ X 106
Where;
N = total number of cells counted
DF = dilution factor
0.4 = volume of the chamber

Leucocytes differential counts

The blood film for the differential leucocytes counts was not too thick nor too thin to ensure a reliable and accurate differential leucocytes count according to the prescription and description of Dacies et al.(2006).

An oil immersion was applied to the stained blood film and observed under the light microscope with X100 objective lens. Longitudinal method was use to count each leucocyte cell against a 100 total count. Myelocytes and metamyelocytes were counted separately and not as neutrophil when encountered. The bands cell counts were given special attention and counted separately too. The differential count was expressed as a percentage of each type of cell and related to the total leucocyte count.

Precaution

Sterile EDTA containers were used to collect the sample to avoid introduction of contaminants. II. llNew hand gloves were used for processing each round of samples.
Each recruited subjects were bleed by vene-punctured
All samples were analysed immediately after collection without delay to avoid remission error
The blood film was not too thin nor too thick to ensure a reliable differential leucocytes count.

Statistical analysis and presentation

The result and the outcomes of each haematological investigations in this study was analysed statistically and presented using method (Chatfield, 1983)

Results

Table1: Paediatric patients with sepsis studied

The patients were enlisted into the study consecutively and prospectively.

Age (yrs)	M (n = 31)	F in= 19)	Total n(%)
Less than 1	16(32)	10(20)	26 (52)
1-5	15 (30)	9. 18)	24 (48)
Grand Total	31 (62)	19(38)	50 (100)

Table 1 : Age and sex distribution of the paediatric patients with sepsis studied.

50 paediatric patients with sepsis of various aetiologies were studied in this programme. These consisted of 16 males (32 percent of the total) and 10 females (20 percent of total) who were less than one year old. The patients who were between one and five years old consisted of 15 males (30 percent) and 19 females (18 percent). On the whole, 31 (62 percent) male patients and 19 (38 percents) females were studies. It could be seen from this observation that baring chance event, paediatric sepsis in the study group discriminated more against males than females. The implication of this finding is discussed in chapter 5.

Table 2: Haematological results of the patients with sepsis studied

Table 2: Packed cell volume, total WBC count and neutrophil lobe count of the patient with sepsis.

Gender	Band Counts (lobes)			n(%)		Total	
	PCV (L/L)	WBC (x109/L)	1-3	4	5+		n(%)
M (n=31)	0.33+0.07		9.9+3.7		22(44) 15(30%)	13(26)	50(100)
F (n=19)	0.35+0.05		9.6+2.8				

Table 4.2 shows that the condition of sepsis impacted more severely on the male than female patients who had lower packed cell volume and slightly higher total white cell count. From this table, it could be seen that 22 patients, representing 44 percent had neutrophil lobe count of between 1-3 i.e. left shift. 15 (30 percent) had four lobes on the average, while 13(26 percent) had five or more lobes, signifying right shift. It is therefore observed that more of the patients had low lobe count (left shift) which will suggest acute infection.

Table 3: Neutrophil segmentation index (NSI) and mean differential leucocytes count result of the patients with sepsis.

Group	NSI	Differential mean leucocytes count (Range mean%)				
	N	L	E	M	B	
Patients	86.6	50-96(88)	3-45(15)	0-8(3)	0-5(1)`	0-1(0)

Table 3: NSI and mean differential mean leucocytes count of patient with sepsis.

Table 4.3 shows neutrophik segmentation index of 86.6% for the patient, for above the reported (Bain.1989) normal value of 16.9% or below, representing an anomaly. The segmentation index was obtained by dividing the number of neutrophiis with frve(13) or more X100) by ihe number of neutrophils with four lobes. Values of greater than 16.9°o are considered abnormal.

Mean differential leucocytes count in the patients showed evidence of reactive neutrophil leueocytasis.

Discussion

In this study, the occurrence of sepsis was a close event between the patients under one year (52%) and those whose ages were between one and a little below five years (48%). 44 percent of the patients had reduced neutrophil segmentation which is consequent on temporary bone marrow stimulation, giving left shift neutropbilia of 1 to 3 lobes. A left shift is reported to be a physiological occurrence in pregnancy (Bain, 1989). But in non-preguard patients, it is reported by this author to commonly indicate a response to infection or inflammation, or some other stimulus to bone marrow. Band cell counts have been employed in the detection of infection in neonates (Akenzua et al., 1974; Christensen et al., 1981). An increase in the number of band cells in relations to mature neutrophils is described as a left shift (Bain, 1989). The under-live paediatric patients in that study who had left shift apparently had the infection as the main stimulus as there was no way of ascertaining of the observed disorder was hereditary.

26 percent of the patients had neutrophil hypersegmentation or right-shift, with the presence of more than 3 percent of five lobed neutrophil (Bain 1989). The clinical usefulness of segmented versus stab neutrophils criteria for differential leucocytes count, particularly in cases of sepsis has been acknowledged (Mathy and Koekpe, 1974). In normal blood, neutrophil have one to five lobes, with six

lobed neutrophils being seen very rarely (Bain, 1989), a rights shift is said to be present if the average lobe counts is increased. The right shift observed in a segment of the patients studied is instructive as sepsis is a serious medical condition with daunting predisposing factors which include malnutrition, prematurity (before 37 weeks of gestation), weak Immune system, heart defect, urinary tract infection, burns or multiple injuries (Levy et al., 2003).

In this study, the 26 percent of the paediatric patients with right-shifts neutrophils had neutrophils segmentation index anomaly (NSI) of 98.5 percent. This value is grossly in excess of the reported normal value of 16.9 percent and below (Bain, 1989). The NSI is sensitive index of hypersegmentation and reflects effects of changes in neutrophil structure on its function. Ezimah (2007), reported neutrophil segmentation index anomaly in the sepsis of acquired immunodeficiency syndrome, just as Davidson (1968) and Bain (1989), reported that right shift or neutrophil hypersegmentation is seen in megaloblastic anaemia and in occasional patients with iron deficiency, infection or uraemia. It also occurs as a rare hereditary characteristic with autosomal inheritance.

The differential leucocytes count for the patients showed a preponderance of neutrophil leucocytosis which is a variation of the expected physiological pattern for the age group.

From this study, it is seen that increased band counts or even of right shift with attendant abnormality of nuclear se observed in the patients may affect neutrophils function and hence mean poor prognosis for the segmentation patients with sepsis.

Conclusion

Under-five patients with sepsis seen in the University of Maiduguri Teaching Hospital either had increased band counts in the majority of cases or rights shift neutrophils with abnormality of segmentation. These findings have grave implications for neutrophil function and subsequently, poor prognosis of the primary condition.-

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How to cite this article:
Ezimah, Anthony C.U., Obeagu, Emmanuel Ifeanyi, Ahmed , Hassan , Ezimah, Uloaku A. and Ezimah, Chinyereugo O. (2016 The prognostic significance of neutrophil polymorph and band counts in under-five children with sepsis in Umth . Int. J. Adv. Res. Biol. Sci. 3(3):68-74.