Evaluation of the Immature Granulocyte Count in the Diagnosis of Sepsis Using the Sysmex XE-2100 Analyser

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Sepsis is a very serious condition in which severe cases can result in shock, disabilities, organ failure and even death [1]. The most important factor in a favourable outcome of this disease is a rapid and accurate diagnosis [2]. The aim of this project was to evaluate if the immature granulocyte count and the calculated IG ratios (% IG / total white cell count) and IT ratios (% IG / total neutrophil count) could be of use as an early indicator of sepsis. A total of 61 patients were included in the study over a four month period. Samples were collected from the Paediatric Intensive Care Unit (PICU), Burns unit and Accident and Emergency departments of Birmingham Children's Hospital (BCH). Any patient undergoing investigation for sepsis or those post operative patients susceptible to sepsis were included in the investigation. The patient's immature granulocyte counts and calculated IG and IT ratios were interpreted in consideration of the patient's clinical notes, blood culture and CRP results. The data was looked at to see if there was any change in the IT or IG ratios. This was statistically compared to the negative control to see if the change in either of these ratios could be used in the paediatric setting for diagnosing sepsis. We found out that the IT ratio was a more sensitive and specific indicator of sepsis than IG ratio or CRP. Therefore through the use of the Sysmex XE-2100 analyser the IT ratio could be used as a fast, inexpensive, reliable indicator of sepsis in a paediatric setting.

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INTRODUCTION

Sepsis is a very serious condition in which severe cases can result in shock, disabilities, organ failure and even death [1]. With over 18 million cases seen each year, it is a major cause of mortality and morbidity worldwide and the 10th leading cause of death in the western world.

What is sepsis?

Sepsis can be defined as "the presence of bacteria or their toxic products in the circulation, encoupled with resultant manifestations" [3].

The discovery of endogenous mediators of host responses has led to the recognition that sepsis is the result of excessive activation of host defence mechanisms, i.e. the body's response to systemic infection rather than the direct effect of the microorganism [4].

The American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine Consensus Committee (SCCM) have described sepsis as a Systemic Inflammatory Response (SIRS) to a documented infection. It is diagnosed when a patient presents with two or more of the symptoms listed in ***Table 1***:

Table 1 *Symptoms in SIRS*

Symptoms in SIRS
Temperature >38˚C or <36˚C
Heart rate >90 beats/min
Respiratory rate >20 beats /min or $paCO_2$ <32mmHg
White blood cell count (WBC) >12 x 10^9/L

Severe sepsis is classified as sepsis with organ dysfunction, hypo perfusion/perfusion abnormalities or hypotension [5].
The most important factor for a favourable outcome of this disease is a rapid and accurate diagnosis [6]. This is not always easy to accomplish since sepsis is such a complex disease and produces a range of non specific clinical symptoms with stages that mimic other disease states. Therefore there are a huge variety of laboratory tests that play a vital role in its diagnosis [7].

Current methods for diagnosis

A number of laboratory tests are currently used in the diagnosis of sepsis including microbiological, biochemical and haematological tests with each of them having individual advantages and disadvantages.

Microbiological tests
The gold standard test is the blood culture, in which 30 mL of the patient's blood is cultured in specific media to examine the potential growth of microbes. This procedure can take up to four days by which time the disease may have had drastic effects for the patient [8].
Also more than 15% of patients [9], which appeared to be clinically septic, had negative blood culture results. This could be due to a number of reasons. Firstly the patient could be septic with bacteraemia but the organisms in the sample did not grow under normal conditions in the culture media [9]. Secondly sepsis can be induced by the products of a microbe. Therefore the apparent septic state may have resulted not from bacteraemia but from cytokine activation either by previous and transient bacteraemia or derived from non-bacterial origin.
Thus, a blood culture is a primary test for sepsis diagnosis, but further tests are needed to both support and aid a quicker diagnosis until the results of this lengthy test are known [9].

Biochemical tests
The acute phase reactants rise in response to inflammation associated with infection, trauma or tissue damage. Biochemical tests such as C-reactive protein measurement (CRP), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra) and circulating intercellular adhesion molecule-1 (cICAM-1) are indicators that an inflammatory response is occurring [10], but are rather non-specific. More accurate tests are needed to reliably diagnose sepsis.

Haematological tests
Haematological tests include the Erythrocyte Sedimentation Rate (ESR), coagulation screens including parameters such as D-Dimers and the full blood count (FBC).

Coagulation parameters are important in the diagnosis of sepsis because a hypercoagulable state can be observed before the onset of the defined clinical signs. However, a hypercoagulable state is not exclusive to sepsis and therefore results from these tests need to be interpreted accordingly as with the biochemical tests [11].

The full blood count is the most common haematological test performed, since in an emergency the results can be available within 15 minutes from a haematology analyzer. The elevation of white blood cell count (corrected for the presences of nucleated red blood cells) is commonly interpreted as evidence of infection. A full blood count measurement is both easily performed and provides helpful diagnostic information; it remains one of the principle tests for sepsis diagnosis and monitoring.

Additional tests
Other diagnostic methods include the medical imaging techniques such as x-rays to rule out pneumonia and CT scans or echocardiograms to detect any organ damage. Although helpful these methods only detect presence of the disease after it has had damaging effects thus is not a useful tool in the rapid and early diagnosis of sepsis [7].

In the last few years many new techniques have arisen which may potentially allow for the early diagnosis of sepsis. These include plasma procalcitonin levels, detection of hypophosphataemia, protein C, leucocytes functional assays, polymerase chain reaction (PCR), erythropoietin and renin, fibronectin, haptoglobin ceruplasmin and transferrin.

All of these latest developments remain in infancy and require specialised skills and equipment. On the whole they are promising but there are some limitations in regard to sensitivity and specificity, they remain laborious, expensive, time consuming and only available in a few centres or primarily as a research tool. A quick economical alternative to help diagnose sepsis is needed before the results of a blood culture are known. The presence of immature granulocytes (IG's) from a full blood count could be one option.
The more recent haematological analysers such as Sysmex XE-2100 have developed more sophisticated methods to allow for the quantitation of immature cell types. It is obtained from a specifically gated area in the DIFF-channel by reporting the absolute immature granulocyte count as well their percentage of the total white cell count [12] and thus allows for a simple, fast and inexpensive quantification of promyelocytes, myelocytes and metamyelocytes. It has been previously described that in sepsis the presence of immature granulocytes in the peripheral blood provides potentially important information indicating enhanced bone marrow activation [13].
A recent study by Nigro et al [14], evaluated the performance of an automated immature granulocyte count as a predictor of neonatal sepsis. They showed that elevated immature granulocyte (IG) counts were associated with positive blood culture results, although with low sensitivity. Other research papers support the fact that the automated IG count may be a useful tool in the diagnosis of sepsis. By dividing the immature granulocyte by the total neutrophil count the so called IT ratio can be calculated. This value may be more specific in diagnosing sepsis since it considers not only absolute numbers of immature granulocytes but compares it to the total neutrophil count. This is potentially important information indicating enhanced bone marrow activation

which is seen in sepsis [15]. An automated IG count offers the potential advantages over microscopy by firstly counting a greater number of cells than the 100 cell differential. Secondly it avoids operator interpretations, and with this is markedly reducing the statistical error. The automated IG count could also provide an indication of early sepsis before the blood culture results are available and guide the clinician into possible earlier treatment.
This project aimed to assess if the Sysmex XE-2100 immature granulocyte count and the calculated IG and IT ratios could be of use as an early indicator of sepsis and whether the immature granulocyte count could provide a faster, reliable and more cost effective method of diagnosis in comparison with other diagnostic tools such as microbiological blood culture or CRP. Overall we aimed to see if the parameters given by the Sysmex XE-2100 can be useful to aid the early diagnosis of sepsis in a paediatric hospital.

MATERIALS AND METHODS

Blood samples

Sample selection
A total of 61 patients were included in the study over a four month period (February-May 2006). Each sample was collected from the Paediatric Intensive Care Unit (PICU), Burns unit and Accident and Emergency departments of the Birmingham Children's Hospital (BCH) under the following selection criteria:

- Patients under clinical investigation of Sepsis
- Post operative patients susceptible to Sepsis e.g. cardiac bypass patients.

Three patients were excluded from the study because they had been referred from another hospital and so suitable follow up samples were unobtainable. The remaining 58 patients were divided into 3 groups (***Table 2***):

Sample collection
Blood samples were obtained by capillary collection into a standard 0.5ml K_3 EDTA sample tube or by venipuncture into standard 2ml K_3 EDTA vacutainers. All samples were sent immediately to the Haematology laboratory via the hospital air tube system and processed within a 90 minute turnaround time from sample receipt.

Methods

A blood smear and full blood count were performed on all patient samples included in the study. The smears were stained with a modified Wright stain on the Hema-Tek® automated slide stainer. The full blood count and automated IG count was performed using the Sysmex XE-2100 haematology analyser.

IT and IG ratios were calculated as follows:

$$\bullet\ \text{IT ratio} = \frac{\text{Total IG count}}{\text{Total Neutrophil count}} \times 100$$

$$\bullet\ \text{IG ratio} = \frac{\text{Total IG count}}{\text{Total White Cell count}} \times 100$$

Results were statistically analysed, compared to a manual differential white blood cell count (WCC) and used for the interpretation of patient data.

Evaluation of immature granulocyte counts and patient notes
The patient's notes were looked at to gain a conclusive diagnosis of sepsis. A comparison between the change in IG and IT ratios, blood culture results and the CRP was made to determine if the IG count could be of use in the diagnosis of sepsis.

1) The blood culture results for each patient were obtained retrospectively for this study.
2) Septic patients with positive blood cultures were assumed to be given the diagnosis of sepsis on the day of aspirate of the first positive blood culture.
3) The full blood count results for 3 days pre diagnosis, the day of diagnosis and 3 days post diagnosis were analysed, where possible, as were the patient's CRP results. All results were interpreted together with patient's notes.

Table 2 *Patient groups*

Group	Patients	Number
1	Diagnosed with sepsis showing a positive blood culture result	31
2	Clinically diagnosed with sepsis showing a negative blood culture result.	7
3	Negative for sepsis	20
		Total 58

Analysis of patient data
The immature granulocyte count and the calculated IG: Mature Neutrophil (IT) ratio was interpreted with data from the patient's clinical notes, blood culture and CRP results.

Establishing a threshold for raised IG and IT ratios
To establish a threshold for raised IG and IT ratios in sepsis, Receiver Operator Curves (ROC) were constructed. The thresholds for both IG and IT ratios were established by taking the values from the curve that gave good sensitivity with the best possible specificity.

Predictive models for both IG and IT ratios in sepsis diagnosis were constructed.

RESULTS AND DISCUSSION

Interpretation of patient's data

The individual IG and IT ratios were calculated for each patient according to the formula depicted above (data not shown). The results showed that a large portion of septic patients had raised ratios, particularly IT ratios with one as high as 37.5. The negative control group, although in some cases raised, did not show a ratio above 3.
A third group of patients was clinically diagnosed with sepsis, but gave negative blood culture results. This was possibly due to antibiotic treatment, or the presence of microbiological products, rather than the organism itself causing the disease. The IG and IT ratios of these patients were statistically compared to the negative control group to see if there is any significant difference, in the same way as with patients with positive blood cultures. All seven of these patients were shown to have raised IG and IT ratios. One patient gave an IT Ratio of over 7.33. This supported the idea that the IT and IG ratios could be of use in sepsis diagnosis.

Establishing a threshold

To establish a threshold for raised IG and IT ratios in sepsis Receiver Operator Curves (ROC) were constructed (***Fig. 1***). They show the false positive rate in relation to 1 minus the false negative rate, i.e. 1-specificity in relation to sensitivity for every possible IG or IT ratio. The corresponding coordinates are listed in the table in ***Fig. 1*** (***B and D***).

An ideal test would be one with a sensitivity and specificity of 100%. From the coordinates of the ROC's in ***Tables 3 and 4***, data does not give a possible cut off with those sensitivities and specificities. Essentially, by choosing a cut off point, a compromise is made between better sensitivity and better specificity. After considering which would be potentially more harmful to a patient, either a false positive or a false negative result, we decided that a test had to have good sensitivity rather than good specificity; because it would be more beneficial to detect that a patient had sepsis as apposed to stating that he does not. Good sensitivity is considered to be anything above 70% [16]. The cut off for IG and IT ratios were selected as 0.35 and 0.65 respectively as at these levels the sensitivity was above 70% showing the best possible specificity.

Comparison of IG and IT ratios

The IG and IT ratios were compared to establish which of the two values would be of most use to aid in the diagnosis of sepsis. ***Tables 3 and 4*** show the predictive models for both, the IG and IT ratios, at their established thresholds. Patients from group 1 (positive blood culture result) and group 2 (negative) are combined as "clinically positive".
Out of the 31 septic patients with a positive blood culture result, 21 (68%) showed a rise in IT ratio above the established threshold and 22 (71%) showed a rise in IG ratios. In the group with negative blood cultures, all 7 patients were shown to have IT ratios above 0.65 $x10^9$/L with 6 out of 7 having raised IG ratios. In comparison, out of the 20 negative control group 7 (30%) were shown to have IG ratios below the threshold and 10 (50%) below the IT ratio threshold. From these predictive models, the sensitivity, specificity, positive and negative predictive values was calculated for each test.

Sensitivity and Specificity

At the IT and IG ratio thresholds of 0.65 and 0.35 respectively, sensitivities were 74% (***Table 5***). These values are above the desired 70% for an accurate test which means that a rise above the threshold in both ratios will detect a significant proportion of patients with sepsis.

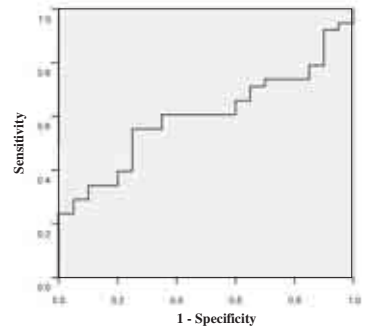
The specificity of the IT and IG ratios at the selected thresholds were 50% and 35% respectively, which is quite poor. The IT ratio showed a considerably higher specificity than the IG ratio, however, the IT ratio would only correctly predict a negative result in 50% of negative cases which is far from ideal. This could be attributed to the small sample size. Further studies with a much larger study group may help to improve the specificity of both the IG and IT ratios.

Positive and Negative Predictive Value

The positive predictive values (PPV) for IT ratio were good at 74%, whereas, the IG ratio had a PPV of 68% (***Table 5***). This showed that the IT ratio is slightly better at indicating sepsis in affected patients. An explanation could be that the IT ratio is calculated solely from the neutrophil count, whereas the IG ratio is calculated from the total white blood cell count (WCC). Furthermore, in sepsis there are vast immune and inflammatory responses, where other white cell populations such as lymphocytes, monocytes, eosinophils and basophils may be effected. An increase in any of these cell types would result in a higher total WCC thus decrease the IG ratio. Therefore, because the IT ratio is calculated totally independent of the other white cell types it was seen to be a more sensitive marker of left shift than the IG ratio.

The IT ratio gave a negative predictive value (NPV) of 50% which was again quite poor, meaning it would only

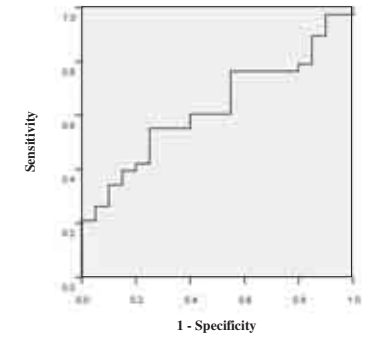
A



B

Positive if greater than or equal to	Sensitivity	1-Specificity
0.2951	0.763	0.85
0.3214	0.737	0.85
0.3289	0.737	0.8
0.3312	0.737	0.75
0.3436	0.737	0.7
0.3561	0.711	0.7
0.358	0.711	0.6
0.3646	0.684	0.65
0.3722	0.658	0.65
0.375	0.658	0.6

C



D

Positive if greater than or equal to	Sensitivity	1-Specificity
0.5413	0.763	0.8
0.5579	0.763	0.75
0.5956	0.763	0.7
0.6224	0.763	0.65
0.6308	0.763	0.6
0.6382	0.763	0.55
0.6501	0.737	0.55
0.6947	0.711	0.55
0.7424	0.684	0.55

Fig. 1 *Receiver operator curves for IG and IT ratios*
The curves show the false positive rate in relation to 1 minus the false negative rate i.e. 1 minus specificity in relation to sensitivity for every possible ratio. Fig. 1 A and 1 B shows the ROC for the IG ratios and the respective coordinates. Marked in red, at 0.35 is the value that gives the best sensitivity and best possible specificity. C and D show the equivalent for the IT ratios, with the threshold for best sensitivity at best possible specificity at 0.65.

Table 3 *Predictive model of the IG ratio. It demonstrates the ability of the ratio to accurately detect if a patient is or is not septic.*

	clinically positive	clinically negative	total
IG ratio >0.35	28 (true positive)	13 (false positive)	41
IG ratio <0.35	10 (false negative)	7 (true negative)	17
total	38	20	58

Table 4 *Predictive model of IT ratios. It demonstrates the ability of the ratio to accurately diagnose sepsis.*

	clinically positive	clinically negative	total
IT ratio >0.65	28 (true positive)	10 (false positive)	38
IT ratio <0.65	10 (false negative)	10 (true negative)	20
total	38	20	58

Table 5 *Positive predictive values, sensitivities and specificities of both the IG and the IT ratios at their established thresholds.*

	senseitivity	specificity	positive predictive value	negative predictive value
IT ratio >0.65	74%	50%	74%	50%
IG ratio >0.35	74%	35%	68%	41%

rule out 50% of disease sufferers. It was however better than the IG ratio with a NPV of just 41%.

The third group of patients (those clinically diagnosed with sepsis showing negative blood culture results), gave 100% PPV, (although a small sample size of 7). This may indicate that the IT ratio would be of great help in diagnosing the small number of patients, who do not have a positive blood culture result. Additional research on a larger sample size is needed to confirm this.

The IT ratio as an indicator of sepsis

From the 20 negative control patients, none had an IT ratio above 3.0. Out of 31 septic patients 6 patients had IT ratios of 4.2 and above. This means that the IT ratio could be used to alert clinicians to start investigations and commence treatment for sepsis in all paediatric patients that have an IT ratio above 4.1.

In agreement with previous studies by Ansari-Lari et al [17] and Nigro et al [15], the IG ratios gave good sensitivity but poor specificity for diagnosing sepsis. The IT ratio, while having poor specificity, had better overall positive and negative predictive values, further supporting the idea that IT ratio would be of most use to aid in the diagnosis. The IT ratio would also be a useful tool in diagnosing those septic patients who have a negative blood culture. Because the IT ratio takes only the neutrophil count into consideration, it would still be raised in those neutropenic patients who develop sepsis, such as those on chemotherapy. The IG ratio takes into account the immature granulocyte count in relation to the total white cell count, and would therefore, be less sensitive to a rise in IG's in those neutropenic patients. Furthermore, an IT ratio ≥4.1 could be used as a good diagnostic indicator for sepsis.

Evaluation of early predictive values of IT and IG ratios

The immature granulocyte count and the calculated IG : total neutrophil (IT) ratios were interpreted with the patient's clinical notes and blood culture results. The full blood count results for 3 days pre diagnosis, the day of diagnosis and 3 days post diagnosis were analysed in septic patients, where possible. The data was examined to see if there was any difference in either the IG or IT ratios up to 3 days pre diagnosis, on the day of diagnosis and up to 3 days post diagnosis. Of the 31 septic patients studied, only 17 patients had 3 days pre and 25 had 3 days post diagnosis samples available. ***Fig. 2*** gives a comparison of IT ratios 3 days pre and 3 days post diagnosis compared with the day of diagnosis.

The results demonstrated a marked increase in the IT ratio with a notable increase in the IG ratio on the day of diagnosis. From the comparison tables (***Tables 6 and 7***) there was little discrepancy between 3 days pre the day and the day of diagnosis. Where 12 (71%) of the 17 patients studied showed an IT ratio ≥0.65 in both samples. Conversely 5 out of 17 (29%) had an IT ratio of <0.65. This suggested that the IT ratio could be used as an early indicator of sepsis, as the increase in ratio can be seen 3 days prior to the day on which the patient was clinically diagnosed with the disease.

For the 3 days post diagnosis samples 18 (72%) of the 25 septic patients studied were shown to have IT ratios above the threshold of 0.65. For the samples taken on the day of diagnosis 17 (68%) of the affected patients had IT ratios >0.65. This means the post sample detected 1 more case. This suggests the IT ratio could be used throughout the course of the disease and with further research has the potential to be used to monitor patient recovery.

Thus the IT ratio showed a notable increase compared to IG ratio in all stages of the disease. This rise can be detected from as early as 3 days pre-diagnosis. Since a blood culture result can take up to 4 days to report, the IT ratio has the potential to indicate sepsis 7 days before the gold standard of microbiology blood culture. Furthermore, a rise in IT ratio ≥4.1 could assist the clinician in a decision to treat with antibiotics in the very

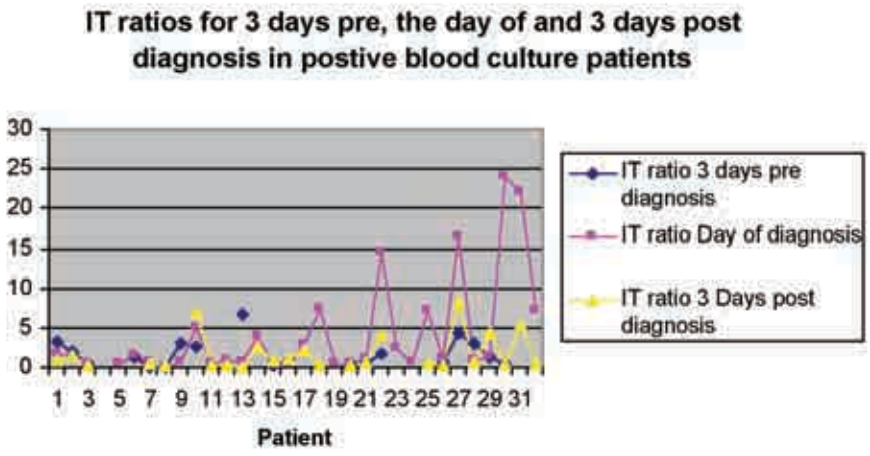


Fig. 2 *IT ratios for 3 days pre, the day of diagnosis and 3 days post diagnosis in positive blood culture patients*

Table 6 *Comparison of the predictive values of the IT ratio on the day of diagnosis, with the IT ratio 3 days pre diagnosis.*

Clinically septic patients			3 days pre diagnosis		
	IT ratio		<0.65	>0.65	total
day of diagnosis	<0.65	count	3	2	5
		%	60%	40%	100%
	>0.65	count	2	10	12
		%	12%	88%	100%
total		count	5	12	17
		%	28%	72%	100%

Table 7 *Comparison of the predictive values of the IT ratio on the day of diagnosis, with the IT ratio 3 days post diagnosis.*

Clinically septic patients			3 days pre diagnosis		
	IT ratio		<0.65	>0.65	total
day of diagnosis	<0.65	count	5	3	8
		%	63%	37%	100%
	>0.65	count	2	15	17
		%	12%	88%	100%
total		count	7	18	25
		%	28%	72%	100%

early stages of the disease, as 17% of the detected patients had an IT Ratio ≥4.1. A rise in IT ratio could also be seen 3 days post diagnosis; which suggests about the possibility that, with further research, it could be used to monitor the effectiveness of treatment.

Are the IT and IG ratios as good as CRP for the diagnosis of sepsis?

The patient's CRP levels were examined because a raised CRP level can be a non specific indicator of sepsis [18]. Each patient's CRP results for 3 days pre diagnosis, the day of diagnosis and 3 days post diagnosis were obtained where possible (these results were only available if the physician had requested the tests). The IT and IG ratios of all groups were then compared to the corresponding CRP. The data was statistically analysed to see if the change in IG or IT ratios are more statistically significant than the change in CRP levels in patients diagnosed with sepsis, compared to the negative control group.

Unfortunately only 21 of the 38 patients diagnosed with sepsis had a CRP requested; 17 of the 20 patients in the negative control group had a CRP request. For this reason the data must be interpreted with caution.

The threshold for a raised CRP is >11mg/L. Of the 21 septic patients studied, 68% showed an increased CRP. However, of the 17 negative control patients 64% were also shown to have raised CRP's. Table 10 compared the CRP to IG and IT ratio. From the calculated values, the sensitivity of CRP for sepsis diagnosis is lower than IT and IG ratios at 71%. The specificity is also very poor at just 35% compared with the 50% seen in the IT ratio. This means that 65% of the patients tested could be miss-diagnosed as septic if a CRP was used alone. The positive predictive values were also better for both, IT and IG ratios, than CRP, with the CRP at 57.7% compared with the 74% and 68% seen in IT and IG ratios respectively. There was only a 57.7% chance that a person would be septic if only a raised CRP result was observed, compared to a 74% chance when a raised IT ratio was seen. With the NPV being the same as IT ratio at 50% it can be concluded that both the IG and IT ratios are better in diagnosing sepsis than CRP. The IT ratio in particular has higher sensitivities, specificities and positive and negative predictive values than CRP.

Limitations

Although the XE-2100 has great potential to aid in sepsis diagnosis it has its limitations. One problem, occurring during sample processing, was the inability of the XE-2100 to give a differential count on certain samples. Some patients with, either very high white cell counts or lipaemic samples such as those seen in liver patients or

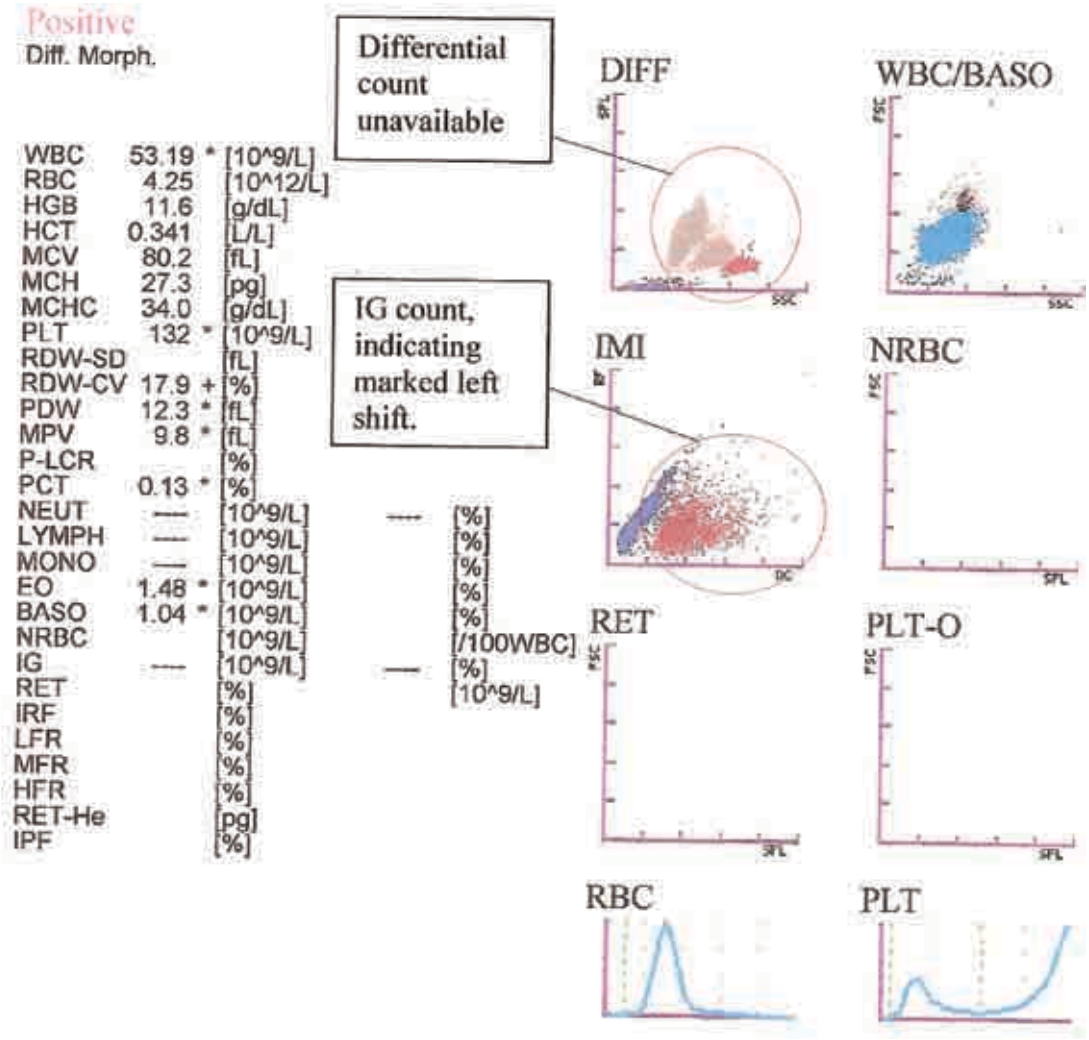


Fig. 3 *Example of XE-2100 showing no differential count*

on Total Parenteral Nutrition (TPN), gave a total but not differential white cell count. An example of this can be seen in ***Fig. 3***. Although a differential count is not given the IMI channel shows a marked left shift indicating a high IG count.

This was attributed to the increased optical density of the sample. The problem was overcome by performing a 1 in 5 dilution with Sysmex cell pack reagent (Sysmex Corporation, Kobe, Japan). Although more costly in time and use of additional reagents, it still remained a faster and cheaper method compared to the manual differential count.

Another limitation of the analyser is again involving turbid samples. The analyser determines the WCC via the WBC and BASO channel and derives the WBC differential through a separate channel called the DIFF channel (which also produces a further WCC which is not used in the final analysis). In a normal sample these white cell counts do not differ significantly. Yet in turbid samples a characteristic wave is seen in the WBC/BASO channel and causes an inaccurate measurement of the WCC. This produces a discrepant count when compared to the WCC generated in the DIFF channel (***Figs. 4 and 5***). Therefore a blood film has to be prepared to determine which count is correct. Although this adds time to the process it still is a faster and cheaper alternative to the manual method.

Time and cost effectiveness

An automated full blood count (FBC) can be performed within ten minutes of sample receipt and costs in the region of £3.00 per test. The CRP has a good turn around time of less than fifteen minutes and costs under £2.00 per test. A blood culture costs £6.17 and can take up to four days to obtain a result. In light of the economics the automated immature granulocyte count is a fast and more cost effective method in diagnosing sepsis. This could improve cost efficiency when treating patients with sepsis in the National Health Service.

CONCLUSION

The results correlated well with other studies [15], showing that both the IG and IT Ratios can be used as a tool in the

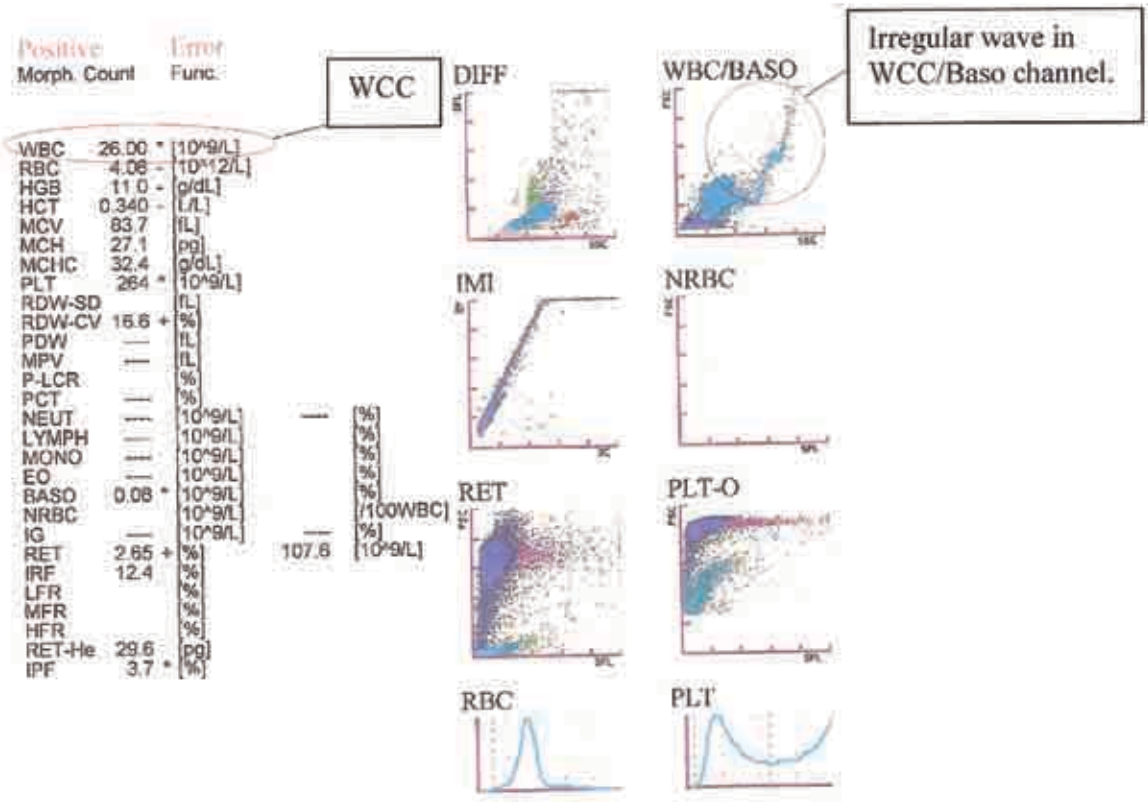


Fig. 4 *Example of an XE-2100 output showing WCC from the WBC/BASO channel*

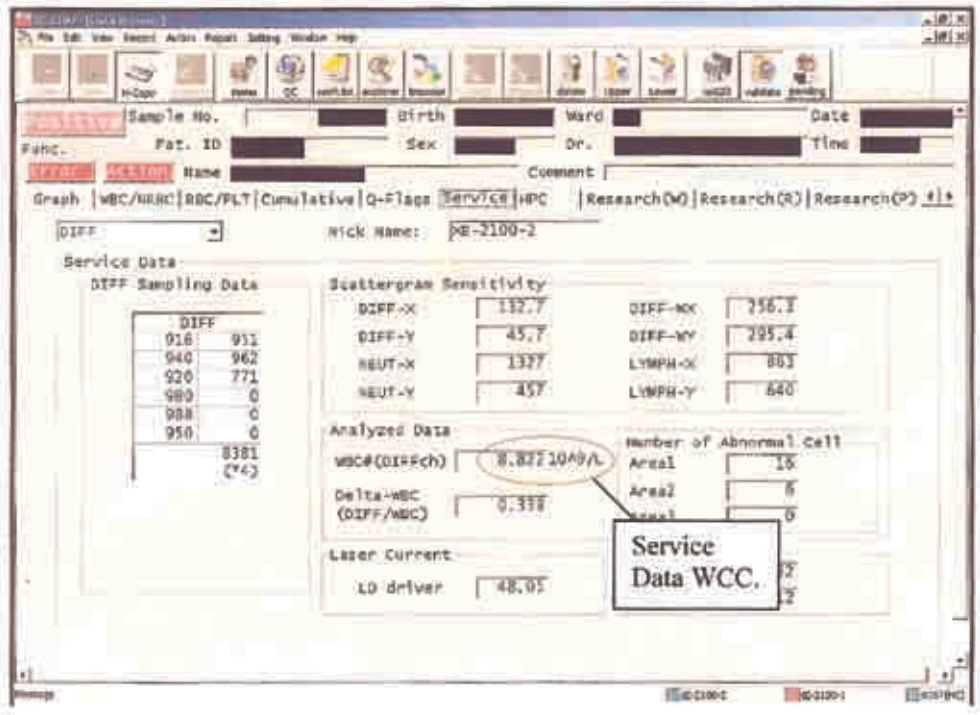


Fig. 5 *Example of the service data output showing the WCC from the DIFF channel (from the same sample)*

early diagnosis of sepsis, although with poor specificity. In addition, the IT ratio could be used to guide clinicians into early treatment. In comparison to the CRP, both IT and IG ratios were found to be beneficial. In the case of blood cultures, a raised IT ratio could be used as an indicator of sepsis when blood cultures are negative due to, either antibiotic treatment or presence of a microbes products rather than a microbe itself. In addition a rise in IT ratio could be detected up to 7 days before blood culture results, making it a good early indicator of sepsis. This not only ensures a more rapid recovery, if treatment is started early, but also has the potential to reduce morbidity and mortality rates. It also may allow patients to be transferred from high dependency wards e.g. ITU to a less labour intensive ward or lead to an earlier discharge. Thus, the IT ratio was a more sensitive and specific indicator of sepsis than IG ratio or CRP. Through the use of the Sysmex XE-2100 analyser the IT ratio could be used as a fast, cost effective, reliable indicator of sepsis in a paediatric setting. However, a larger study would be needed to confirm the findings of this project and perhaps increase the specificity. Further studies on neonates, adult groups and neutropenic patients may also provide some support to the concept that the IT ratio could be used to aid in the diagnosis of sepsis.

References

1) *Angus DC. Linde WT, Cortlet J : Epidemiology of severe sepsis in the United States. Critical Care medicine, 29 (7) : 1303-1310 , 2001.*
2) *Perl TM, et al.: Long term survival and function after gram negative sepsis. JAMA , 274 (4) : 338-345,1995.*
3) *Bernard GR : A recent definition of severe sepsis. New England Journal of Medicine , 344 : 699-709 , 2001.*
4) *Balk RA : Severe sepsis and septic shock. Critical Care Clinics ,2 : 179-192 , 2000.*
5) *American College of Chest Physicians/Society of Critical Care Medicine. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Critical Care medicine , 20 : 864-874 , 1992.*
6) *Reinhart K, Meisner M : Update on the diagnosis of sepsis. European Society of Anaesthesiologists. Anaesthesiology and intensive care medicine , 2002.*
7) *Brun-Boison C, Doyan F, Corlet J : Incidence risk factors and outcomes of sever sepsis and septic shock in adults. JAMA , 274 (12) : 968-974 , 1995 .*
8) *National committee for clinical laboratory standards (NCCLS) . Reference leucocyte differential counts NCCLS document H20-A 1, 1992.*
9) *Von Landeberg P, Shoenfield Y : New approaches in the diagnosis of sepsis. IMAJ , 3 : 439-442 , 2001.*
10) *Farooqui S, et al.: Evaluation of C-reactive protein as an early indicator of blood culture positivity in neonates. Pakistan Journal of medical sciences, 21 (1): 69-73 , 2005.*
11) *Bochud PY, Calandra T : Pathogenesis of sepsis: New concepts and implications for future treatment. British Journal of Medicine , 326 : 262-266 , 2003.*
12) *Weiland T, Kalkman H, Hein H : Evaluation of the automated immature granulocyte count (IG) on sysmex XE-2100 Automated analyser vs. visual microscopy(NCCLS H20-A). Sysmex Journal Int., 12 : 63-70 , 2002.*
13) *Heklotz R, Huber AR : Precision and accuracy of the leukocyte differential on the Sysmex XE-2100. Sysmex Journal Int., 11 : 8-21 , 2001.*
14) *Nigro KG, et al. : Performance of an automated immature granulocyte count as a predictor of neonatal sepsis. American Journal of Clinical pathology , 123 (4) : 618-24 , 2005.*
15) *Cenci A, Maconi M, Casolari B : Evaluation of the diagnostic performance of the Sysmex XT-2000i Automated Haematology analyser in the detection of immature granulocytes. Sysmex Journal Int., 15 (1):1-6 , 2005.*
16) *Loong T.W : Understanding sensitivity and specificity with the right side of the brain. British Medical Journal , 327 : 716-719 , 2003.*
17) *Ansari-Lari MA, Kickler TS, Borowitz MJ : Immature granulocyte measurement using the Sysmex XE-2100. Relationship to infection and sepsis. American Journal of clinical pathology , 120 (5) : 795-759, 2003.*
18) *Ainbender E, Cabata EE, Guzmann DM : Serum C-reactive protein and problems of the newborn infant. Journal of paediatrics, 101 : 438-40, 1982.*