

Diagnosis of spontaneous bacterial peritonitis: An update on leucocyte esterase reagent strips

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Abstract

Ascites remain the commonest complication of decompensated cirrhosis. Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid (AF) in the absence of a contiguous source of infection and/or an intra-abdominal inflammatory focus. An AF polymorphonuclear (PMN) leucocyte count $\geq 250/\text{mm}^3$ -irrespective of the AF culture result- is universally accepted nowadays as the best surrogate marker for diagnosing SBP. Frequently the results of the manual or automated PMN count do not reach the hands of the responsible medical personnel in a timely manner. However, this is a crucial step in SBP management. Since 2000, 26 studies (most of them published as full papers) have checked the validity of using leucocyte esterase reagent strips (LERS) in SBP diagnosis. LERS appear to have low sensitivity for SBP, some LERS types more than others. On the other hand, though, LERS have consistently given a high negative predictive value ($> 95\%$ in the majority of the studies) and this supports the use of LERS as a preliminary screening tool for SBP diagnosis. Finally, an AF-tailored dipstick has been developed. Within the proper setting, it is set to become the mainstream process for handling AF samples.

INTRODUCTION

Ascites remains the commonest of the three major complications of advanced or decompensated cirrhosis (along with hepatic encephalopathy and variceal haemorrhage). Cirrhotics with ascites have, over a one-year period, 10% probability of developing the first episode of spontaneous bacterial peritonitis (SBP)^[1]. Conn first introduced the term SBP, publishing his clinical findings just one year after Kerr *et al* described (in 1963) 11 cases of seemingly unexplained infection of the ascitic fluid (AF)^[2].

SBP is defined as the infection of AF in the absence of a contiguous source of infection and/or an intra-abdominal (and potentially surgically treated) inflammatory focus. Depending on the patient population examined (outpatients or hospitalised), the prevalence of SBP varies from 3.5% and 30%^[3]. Around 50% of SBP episodes are present at the time of hospital admission, whilst the remainder are acquired during the hospitalisation period^[2]. The mortality of untreated SBP remains high ($> 80\%$), and a satisfactory patient course and clinical outcome is based on an aggressive approach aiming to rapid diagnosis and prompt initiation of antibiotic therapy.

DIAGNOSIS OF SBP

The clinical manifestations of SBP can be subtle and in-

sidious, and its diagnosis requires a high index of clinical suspicion. Abdominal paracentesis is considered necessary for all patients with ascites on hospital admission, in-patient cirrhotics with ascites who develop clinical signs of sepsis, hepatic encephalopathy, (sudden or unexplained) renal impairment and/or all cirrhotics who develop GI bleeding^[4]. Unfortunately, a clinical diagnosis of infected AF without a paracentesis is not adequate^[1].

An AF polymorphonuclear (PMN) leukocyte count $\geq 250/\text{mm}^3$, irrespective of the AF culture result, is universally accepted nowadays as the best surrogate marker for diagnosing SBP^[5]. The presence of positive AF cultures is confirmatory, but by no means a necessary prerequisite for instigation of antibiotic therapy. In fact, it is considered a “fatal” mistake to wait 48 h for culture results before initiating therapy, where it is indicated.

Frequently the results of the manual PMN count do not reach the hands of the responsible medical personnel in a timely manner^[6]. Such situations include busy night or weekend shifts, small hospitals with off-site laboratory facilities, or units with limited case-load and liver disease expertise. We have recently showed that the mean delay from paracentesis to a validated PMN result out-of-hours was more than 4 h^[7]. Furthermore, manual AF PMN counting is laborious and costly. The use of automated cell counters has now been backed-up by sufficient published evidence to become the common practice^[5,8].

However, even automated cell counts suffer generally from similar constraints to those described above for manual techniques. Therefore, any alternative test that may provide or, more importantly, exclude a diagnosis of SBP at the bedside and reduce the “tap-to-first shot” time is considered welcome. The leukocyte esterase reagent strips (LERS), commonly used in every day practice for the rapid diagnosis of urinary tract infections (UTIs), were certainly featuring as a promising candidate.

LERS IN SBP

LERS had already been successfully evaluated in the diagnosis of infection in other sterile body fluids i.e. synovial, pleural, cerebrospinal fluid and peritoneal dialysate^[9-11]. The LERS test is based on the esterase activity of the leucocytes. A pyrrole, esterified with an amino-acid is used as the substrate; hydrolysis of the ester (mediated by the esterase) releases the pyrrole which in turn reacts with a diazonium salt yielding a violet or purple azo dye in the relevant pad of the strip^[11]. LERS are not specific for PMNs and the interpretation of the colorimetric reaction is inherently subjective, therefore the method is considered qualitative or semi-quantitative at best. Butani *et al*^[12] were the first to present their results on the use of LERS in SBP diagnosis as an abstract in DDW 2000.

Since then, 26 publications followed (23 as full, peer-reviewed papers and 3 as either an abstract or a letter to Editor; of them, 22 are in English, 2 in French, 1 in Chinese & 1 in Korean), with the first full paper that of Vanbiervliet *et al*^[13] validating the Multistix[®]8SG.

Their results were very encouraging. Thus, various

LERS were eventually validated in what were mostly single or two-centre studies (Table 1), with one notable exception in the French multicentre study (Nousbaum *et al*^[28], 70 centres) published initially as an abstract and later as a full paper in 2007. It is important to note here that the grading is different for each dipstick, and therefore the cut-off leucocyte count should be used instead, in order to draw meaningful conclusions.

The French multicentre study pointed out the weakness of Multistix[®]8SG and, to a certain extent, of the concept of using dipstick in SBP diagnosis overall. Furthermore, 2 systematic reviews^[9,10] have been published in 2008, both pointing out that the heterogeneity in the number of patients included in each study, the AF samples tested and SBP episodes observed, as well as in all measures of LERS performance, did not allow pooling of the results via meta-analysis. Overall, the Aution[®] and Combur[®] dipsticks have performed better^[38] (in regards to the negative predictive value) than the Multistix[®]. The spectrophotometric analyser Clinitek[®] 50, compatible with the Multistix[®] dipstick, was used in only 6 studies.

The rather intense research on the field has brought up important details on the limitations of LERS. First, the results seem to be influenced by the number of PMNs in the AF, LERS performing less well if the PMN count $< 1000/\mu\text{L}$ ^[39]. Second, all LERS validated in the SBP studies were initially designed for use in the diagnosis of UTIs; in infected urine though, both the number of leucocytes and the protein content are quite different, the first being significantly higher than in most SBP^[39], while the latter does not exceed the 1 g/L level^[35]. The above 2 factors are considered significant for the observed low sensitivity of some LERS. I need to mention again here that, aside the fact there is significant inter-study variability in terms of the LERS brands used, as well as to the cut-off level examined, LERS are not specific for PMNs and the interchangeable use of PMNs and leucocytes (seen in the majority of the studies) is confusing to the reader. Finally, LERS are not suitable for the few cases of chylous ascites or peritoneal tuberculosis.

On the other hand, LERS have consistently given a high negative predictive value (NPV) of above 95% in the majority of the studies and, as in SBP, a false positive result (which might eventually lead to the ‘adverse’ administration of a single dose of an overall well-tolerated antibiotic^[28]) is considered ethically and medically acceptable advocating the use of LERS as a preliminary screening tool for SBP diagnosis. In addition, Castelote *et al*^[33] only recently showed that LERS, despite their qualitative nature, could be well used in the clinical management of SBP. The low cost of the strips can only be considered a significant advantage.

Only one study has checked the combine use of the LERS with the relevant pad for nitrites. There was no additional advantage by combining the two results. Finally, despite clear evidence to support its use^[5], no study has validated the combination results of LER pad with that of the pH^[3].

CONCLUSION

In conclusion, there is reasonable amount of evidence

Table 1 Studies, patients included, ascitic fluid samples tested, inpatients/outpatients, type of leukocyte esterase reagent strips used, leukocyte esterase reagent strips cut-off grade of the study with Sens, Spec, PPV and NPV

Study	Patients	Samples	In/Out	M/F	SBP	LEERS	LEERS cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Vanbiervliet <i>et al</i> ^[13]	72	78	72/0	44/28	9	Multistix8 [®] SG	70 leuc/μL-G2	100	100	100	100
Castelote <i>et al</i> ^[14]	128	228	128/0	91/37	52	Aution [®] sticks	75 leuc/μL-G2	96	89	74	99
Thévenot <i>et al</i> ^[15]	31	100	23/8	13/18	9	Multistix8 [®] SG	125 leuc/μL-G3	89	100	100	99
						Combur2LN [®]	75 leuc/μL-G2	89	100	100	99
Butani <i>et al</i> ^[16]	75	136	n/s	n/s	12	Multistix10 [®] SG	70 leuc/μL-G2	83	99	91	98
Sapey <i>et al</i> ^[11]	34 (s-group)	55 (s-group)	n/s	51/15	13	Multistix10 [®] SG	25 leuc/μL-G1	83/100	96/100	83/100	96/100
	76	184				Nepheur-test [®]	25 leuc/μL-G1	86/100	92.5/100	75/100	99/100
Sapey <i>et al</i> ^[17]	51	245	9/42		17	Multistix10 [®] SG	25 leuc/μL-G1	64.7	99.6	91.7	97.4
						Nepheur-test [®]	25 leuc/μL-G1	88.2	99.6	93.8	99.1
Kim <i>et al</i> ^[18]	257	257	257/0	187/70	79	UriSCAN [®]	75 leuc/μL-G2	100	99	98	100
Kim <i>et al</i> ^[19]	53	75	53/0	36/17	18	Multistix10 [®] SG	75 leuc/μL-G2	50	100	100	87
						UriSCAN [®]	75 leuc/μL-G2	100	100	100	100
Sarwar <i>et al</i> ^[20]	214	214	214/0	116/98	38	Combur10 [®]	75 leuc/μL-G2	95	92	72	99
Wisniewski <i>et al</i> ^[21]	47	90	47/0	27/20	6	Multistix8 [®] SG	15 leuc/μL-G1	83	83	42	97
Braga <i>et al</i> ^[22]	42	100	35/7	10/32	9	Combur [®] UX	75 leuc/μL-G2	100	98.9	92.3	100
Rerknimitr <i>et al</i> ^[23]	127	200	106/21	75/52	42	Combur10M [®]	25 leuc/μL-G1	88	81	55	96
Campillo <i>et al</i> ^[24]	116	443	n/s	76/40	33	Multistix8 [®] SG	70 leuc/μL-G2	45.7	98	75	93.3
						Combur2LN [®]	75 leuc/μL-G2	63	99.2	91	92.9
Li <i>et al</i> ^[25]	84	84	84/0	47/37	25	Multistix10 [®] SG	15 leuc/μL-G1	92.8	84.7	71.8	96.1
Ribeiro <i>et al</i> ^[26]	106	200	80/26	82/24	11	Multistix10 [®] SG	15 leuc/μL-G1	86	96	60	99
Gaya <i>et al</i> ^[27]	105	173	71/34	71/34	17	Multistix10 [®] SG	15 leuc/μL-G1	100	91	50	100
Nousbaum <i>et al</i> ^[28]	1041	2123	686/355	748/293	117	Multistix8 [®] SG	70 leuc/μL-G2	45.3	99.2	77.9	96.9
Torun <i>et al</i> ^[29]	63	63	63/0	38/25	15	Aution [®] sticks	75 leuc/μL-G2	93	100	100	98
Nobre <i>et al</i> ^[30]	55	109	55/0	33/22	9	H-T Combina [®]	75 leuc/μL-G2	78	88	37	98
de Araujo <i>et al</i> ^[31]	71	155	43/28	57/24	17	Multistix10 [®] SG	15 leuc/μL-G1	80	98.5	90.9	96.2
		159				Choceline 10 [®]	75 leuc/μL-G2	76.9	97.7	87	95.6
Balagopal <i>et al</i> ^[32]	175	n/f	n/f	146/29	n/f	Magistix10 [®]	125 leuc/μL-n/f	92	100	n/f	n/f
Castellote <i>et al</i> ^[33]	51	n/s	51	n/s	53	Aution [®] sticks	75 leuc/μL-G2	89	86	62	97
Rerknimitr <i>et al</i> ^[34]	143	250	n/s	91/52	30	Multistix10 [®] SG	?25 leuc/μL-G1	80	94.5	66.7	97.2
						Aution [®] sticks	?250 leuc/μL-G3	90	93.2	64.3	98.6
						Combur10 [®]	?75 leuc/μL-G2	90	93.2	64.3	98.6
[letter]Gülberg <i>et al</i> ^[35]	n/s	194	n/s	n/s	16	Multistix10 [®] SG	n/s	31	n/s	n/s	n/s
						Combur [®]	n/s	44	n/s	n/s	n/s
[letter]Farmer <i>et al</i> ^[36]	256	311	n/s	161/95	59	Multistix8 [®] SG	70 leuc/μL-G2	96	96.5	90.7	99.4
[abstract] Delaunay-Tardy <i>et al</i> ^[37]	n/f	n/f	n/f	n/f	n/f	Multistix8 [®] SG	n/f	60	n/f	n/f	n/f

Sens: Sensitivity; Spec: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; LEERS: Leucocyte esterase reagent strips; SBP: Spontaneous bacterial peritonitis; n/s : Not stated; n/f : Not found; G : Grade (as per LEERS); In/out: Inpatients/outpatients; M/F : Male/female; s-group: subgroup

to support the use of LEERS in the work-up of patients suspected of having SBP. The PMN count (be it manual or automated) is not to be abolished from SBP diagnostic algorithm. Remote hospitals, less affluent health systems and busy junior clinicians should realise the benefit of LEERS and incorporate them in their AF handling routine. A “new kid on the block” has just appeared^[40] in the race against SBP; if further validation studies worldwide are supportive, it is set to become the mainstream process for handling AF samples^[41].

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