Urine microscopy and infection in general practice

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SUMMARY. To test the value of urine microscopy 100 consecutive specimens were examined in the surgery and the results correlated with the subsequent laboratory culture reports.

An assessment of the degree of pyuria was made by low power microscopy of a thick drop of fresh urine. A second specimen was examined under high power for the presence or absence of motile bacilli. The techniques used are described and quantified.

The laboratory report was definitive in 88 of the 100 cultures. All the 33 specimens with 10^5 bacteriuria had some degree of pyuria and in 27 (82 per cent) motile bacilli had been found. In the 50 with no significant bacteriuria no motile bacilli had been seen in 38 (76 per cent).

In these 88 specimens a diagnosis made in the surgery based entirely on bacterial microscopy would have been correct in 80 per cent, combined with cytological microscopy in 87 per cent, and with the addition of clinical features in 92 per cent.

In the remaining 12 cases the laboratory report was inconclusive and would have made no difference to my conclusions.

Introduction

IN general practice, complaints suggestive of urinary tract infection are common and its exclusion is often necessary in the elucidation of such conditions as pyrexia of unknown origin, abdominal pain, haematuria, or turbid urine specimens proffered by diabetic and antenatal patients. Also, when following a course of treatment for an infection it is useful to have some objective test of cure to augment the patient's own opinion. A sample of urine can be sent to the laboratory for examination and culture and the result will arrive in several days, but this is little help to the doctor who has to make a clinical decision while the patient is still in his consulting room.

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For 25 years I have relied on urine microscopy to overcome this problem, at first by looking at the cells but for the past 10 years seeking motile bacilli too. When our local public health laboratory began sending its reports on photocopies of the culture request forms it offered a simple means of checking the validity of this reliance.

Method

The series consisted of 100 consecutive specimens of urine collected in the surgery from patients in which culture of the urine was considered desirable.

Collection of specimens

Each patient was supplied with a covered sterile kidney dish and a gallipot containing two sterile cotton wool swabs, one for cleaning the introitus and the other for plugging the vagina or in the case of males for wiping the glans after retraction of the prepuce. The patient was asked to collect a mid-stream specimen of urine if possible.

Low power microscopy specimen. A 6 mm diameter glass rod kept in a glass cylinder containing several inches of one per cent hypochlorite solution was rinsed under the tap and used to stir the urine before transferring a thick drop to a microscope slide.

High power microscopy specimen. Another drop of urine was transferred to a second slide and was covered by lowering a cover slip without compression.

Laboratory specimens. A Mackey and Sandys' (1965) culture spoon was dipped in the urine and returned to its bottle. A plain tube was three quarters filled with urine. These specimens were kept at room temperature until it was convenient to deliver them to the laboratory, usually within 24 hours.

Recording

The two laboratory specimens were accompanied by the standard form from the public health laboratory ser-

vice, and on one half of this the clinical details and the urine microscopy findings were recorded. In due course a photocopy of the entire form was returned with the laboratory findings on the other half thus affording a ready means of comparison. These reports were kept and provided the information on which this report is based.

Thick drop, low power microscopy

The microscope and its light were kept ready set up beside the sink with the substage condenser racked low. The specimen was viewed through a x10 eyepiece and 2/3 objective lens which provided sufficient magnification (x110) to differentiate between the various types of cell. Several fields towards the centre of the drop were scanned with slight racking up and down according to the thickness of the drop. This process took about half a minute. The following features were noted:

Epithelial cells. A high proportion of polygonal squamous cells was taken to indicate an unsatisfactory specimen due to vaginal contamination.

White blood cells were recorded as 0 (<1 cell per low power field (LPF)), ± (one to five per LPF), + (six to 10 per LPF), + + (many per LPF), and + + + (very many per LPF).

Red blood cells were recorded similarly. A substantial number of red blood cells rendered the assessment of the number of white blood cells problematical and *vice versa.*

High power microscopy

This was performed with a x10 eye piece with 1/6 objective lens giving a magnification of x490. Due to the rapid movement of urine induced by the lowering onto it of the cover slip it was necessary to leave it for a minute or so to become still. The purpose of this examination was the identification of motile bacilli which are the pathogenic organisms in 95 per cent of urinary infections in general practice (Black, 1972). These bacilli may be easily visible if long or linked together in strings; or they may be at the limits of resolution but these smaller organisms are slightly elongated and usually in rapid revolution thus attracting attention. To find them it may help to bring a cell sharply in focus and search in the neighbourhood. At least one motile bacillus per high power field was the minimum requirement for diagnosing bacteriuria.

Bacteria may also be seen attached to the cell membrane of leucocytes and if numerous enough and motile they may impart a slight rotary twitching movement to the phagocyte. These cells were called Medusae and their presence noted.

This procedure required more time and care and usually took a minute or so unless the urine was teeming with large bacilli when a glance was sufficient for diagnosis.

Laboratory technique

The urine specimen was centrifuged and the deposit examined under a cover slip. The number of cells reported was an empirical one derived from the number of cells per high power field.

The quantitative cultures were reported:

"Significant growth"	>100,000 organisms/ml
"Equivocal growth"	10,000 to 100,000 organisms/ml
"Picture obscured by contamination"	Mixed growth of organisms
"No significant bacteriuria"	<10,000 organisms/ml

Results

Thick drop microscopy

The scanning assessment of the number of white blood cells is compared with the mean laboratory cell count (Figure 1). The near linear relationship conceals a number of individual disparities.

In Table 1 the assessment of white blood cells is correlated with the quantitative laboratory culture and leucocyte count. The only noteworthy finding was that thick drop microscopy revealed the presence of white blood cells in every one of the 33 specimens which the laboratory subsequently reported as having significant bacteriuria. Laboratory microscopy confirmed the presence of leucocytes in 32 of these. In the single exception microscopy in the surgery had revealed numerous white blood cells suggesting either considerable cell degeneration in the specimen or an error.

Figure 1. *Thick drop white blood cell assessment and number of specimens: mean laboratory cell count.*

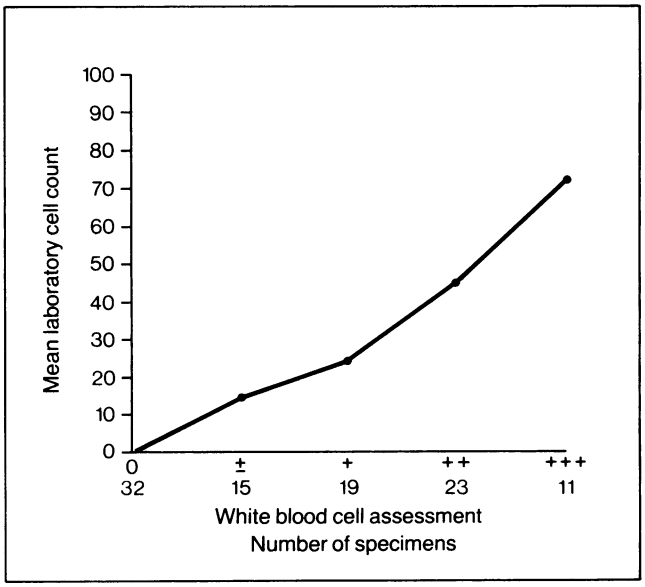

Table 1. Thick drop estimate of white blood cells per low power field: laboratory culture and count of white blood cells.

	Number of cases	0 <1 WBC	± 1 to 5 WBC	+ 6 to 10 WBC	++ Many WBC	+++ Numerous WBC	Mean laboratory leucocyte count
Significant growth ($>10^5$)	33	0	5	7	12	9	42
Equivocal growth	9	1	2	3	2	1	36
Picture obscured by contamination	8	4	2	1	1	0	31
No significant bacteriuria ($<10^4$)	50	27	6	9	7	1	11
Total number of cases	100	32	15	20	22	11	
Laboratory mean cell count		1.3	14.2	25.8	45.6	72.7	

WBC white blood cells.

There was little correlation between the degree of pyuria and the cultural results, and leucocytes were present in 23 of the 50 specimens found to have no significant bacteriuria.

High power microscopy

The quantitative culture resulted in a definitive report in 83 out of the 100 specimens: 33 with "significant growth" and 50 with "no significant bacteriuria". It will be seen (Table 2) that motile bacilli were noted as being present in 27 (82 per cent) of the former and absent in 38 (76 per cent) of the latter. Of the six cases with significant bacteriuria in which no motile bacilli were seen on microscopy, all had pyuria, one with Medusae, and two had erythrocytes while, clinically, three had renal pain and one a fever. The causative organisms were non-motile *(Staphylococci)* in three cases. On the other hand it has to be confessed that of the 27 cases with significant bacteriuria in which motile bacteria were seen, four of the cultures were of non-motile organisms also *(Klebsiella, Micrococcus* and two *Staphylococci).* Presumably, this was due to seeing contaminating motile bacilli or seeing the pathogens in movement imposed by urine currents.

Of the 12 cases with no significant bacteriuria in which motile bacilli were seen on microscopy, five had no pyuria and one had numerous epithelial cells with white blood cells, suggestive of gross vaginal contamination. All six of these would be highly suspect.

Medusae were noted in 17 cases, 10 with significant and two with equivocal infections, one with contamination and four with no significant bacteriuria.

Out of the 88 cases in which the laboratory was able to make a definitive diagnosis of either significant bacteriuria or no significant bacteriuria a decision based entirely on the finding or not finding of motile bacteria would have been wrong in 18 cases (20 per cent). Taking into consideration the clinical and cytological features mentioned, the provisional diagnosis at the time of consultation would have been incorrect in seven cases (eight per cent).

In the 17 cases in which the laboratory reported either an "equivocal growth" or "picture obscured by contamination" little help would have accrued if the clinical decision making had been deferred until the arrival of the report. With the help of urine microscopy as well as the clinical features a decision at the time to treat 12, have a further specimen in two, and to await the laboratory report in three would at least have some basis of rationality (Table 3).

Table 2. High power microscopy of motile bacilli: quantitative laboratory culture. (Percentages in brackets).

	Number of cases	Motile bacilli seen	No motile bacilli seen
Significant growth	33	27 *(82)*	6 *(18)*
No significant bacteriuria	50	12 *(24)*	38 *(76)*
Equivocal growth	9	4	5
Picture obscured by contamination	8	6	2

Quantification

The results reported above and my use of this technique over the years has been entirely empirical so that it seemed desirable to quantify what was observed.

Two specimens, A and B, of fresh sequestrated blood were obtained from the laboratory with the total cell counts recorded by Coulter counter. These were suitably diluted with isotonic saline to provide a series of suspensions of known cell content. Those of 100,000/ml or less were optically clear.

From each suspension thick drops were placed on five slides. The number of cells per low power field was either assessed or counted in five fields well within the edge of each drop. Cover slips were then lowered onto these drops for high power microscopy and the cells in 10 fields counted on each slide. The mean cell counts together with scanning assessments are given in Table 4.

The thick drop technique detected cell counts down to five per cubic millimetre which was equivalent to

Table 3. Culture "Equivocal growth" or "Obscured by contamination": clinical features, urine microscopy, and management decision at the time of consultation.

Clinical features			Urine microscopy				Decision	Culture report
Frequency	Dysuria	Other	Red blood cells	White blood cells	Epithelial cells	Motile bacilli		
+	+	Fever, fifth day puerperium	+	+M		+	Treat	Equivocal growth
+	+	Loin ache	+	++M		+	Treat	
+	+			++		+	Treat	
+	+		+	+++		+	Treat	
+	+		±	+		0	Treat	
+	+			±		0	Treat	
+	+			±		0	Treat	
	+			+	±	0	Treat	
	+	Previous infection			+	0	FS	
+				++M		+	Treat	Obscured by contamination
+	+	Fever, rigors	±	±		+	Treat	
		Incontinence, offensive urine, dementia		±		+	Treat	
+	+	Backache		+		0	Treat	
		Offensive urine				+	FS	
		Night sweats			+	+	0	
		Turbid antenatal urine			+	+	0	
		Enuresis		±	±	0	0	

M Medusa cells.
0 Await laboratory report.
FS Further specimen collected.

Table 4. Known blood cell concentrations: high and low power cell counts and assessments.

	Low power, thick drop				High power, cover slip	
	Cell counts and assessments per field				Cell counts and assessments per field	
Cells/ml	Blood suspension A		Blood suspension B		Blood suspension A	Blood suspension B
10^8		+++		+++	+++	+++
10^7		+++		+++	+++	+++
10^6		+++		+++	5.2	7.8
500,000		+++		+++	5.4	2.9
100,000		++	68	++	0.9	0.1
50,000	23	++	23	++		
25,000	3.4	±	6.3	+		
10,000	3.1	±	3.5	±		
5,000	1.9	±	1.4	±		

between one and two cells per low power field.

The high power technique is evidently incapable of detecting 10^5 bacilluria, might possibly detect 10^6, but should readily detect 10^7 or greater. There was often a marked disparity in the cell counts of adjacent fields and viewing the same film under low power revealed the reason for this: the cells were distributed in an erratic pattern often leaving lacunae more than equivalent to a high power field. Plainly, in seeking motile bacilli in urine, numbers of contiguous fields should be scrutinized.

Discussion

At room temperature the cells in urine remain intact for at least eight hours (Houghton and Pears, 1957) but they have largely degenerated by three days if the pH rises to nine (Porter and Brodie, 1969). After centrifuging at 2,500 rpm for five minutes only half the red and white cells remain intact (Gadeholt, 1964). Fresh urine microscopy avoids these sources of error but substitutes that which is due to the variation in thickness of the drop.

Most normal urines contain fewer than five leucocytes per cubic millimetre (Moore *et al.*, 1965) which is equivalent to one to two cells per low power field on thick drop microscopy. Patients with asymptomatic bacteriuria may have few or no more cells than this (Williams *et al.*, 1965; Savage *et al.*, 1969). On the other hand Mond and colleagues (1965) found in 43 patients with symptoms as well as bacteriuria that all had more than 10 white cells per cubic millimetre although an excess was also present in 47 per cent of those with no significant infection. In this series, of the 33 patients with bacteriuria 28 had symptoms referable to the urinary tract and a further one had abdominal pain. All of them had pyuria on thick drop microscopy but so did 23 of the 50 patients with no infection.

The practical conclusion is that a patient with urinary tract symptoms and no pyuria on fresh urine scanning is highly unlikely to be revealed as having bacteriuria when the culture report arrives. On the other hand such a patient with pyuria has only a one-in-two chance of having a significant infection.

Kunin (1961) prepared artificial suspensions of various bacteria in normal urine and in the case of *E. coli* found no organisms in uncentrifuged specimens with 10^5/ml, rare or questionable organisms per high power field (HPF) in 10^6, one to 100 per HPF in 10^7, and innumerable loosely packed organisms per HPF in 10^8. Of 55 patients with significant bacteriuria 44 (80 per cent) had concentrations of 10^6 or greater and 32 (58 per cent) of 10^7 or greater. In this series of 33 infected urines motile bacilli were seen in 27 (82 per cent).

Goldberg and colleagues (1965) found that in six patients with significant bacteriuria and forcing fluids, serial samples collected by suprapubic bladder aspiration all demonstrated a fall in the degree of bacteriuria, in one case from 10^8 to 10^2 in two hours. Most patients with symptoms of urinary tract infection drink more on their own initiative and they almost always micturate before setting out for the doctor's surgery with the fresh sample of urine which they know will be asked for. For the purposes of this investigation a further sample of urine was collected in the surgery and in several cases there was a considerable reduction in the number of cells and motile bacilli seen in the second specimen.

Dysuria and frequency are unpleasant symptoms. If in addition to these there is pyuria, either alone or with haematuria, it can be assumed that there are inflammatory changes in the urethra, bladder, or higher. Relief of the symptoms is speeded by antibacterial treatment and recovery is associated with disappearance of the cells from the urine. Bacteriuria can be asymptomatic but if it causes inflammation of the urinary tract it will cause pyuria and usually symptoms also. Stamey and colleagues (1971) have demonstrated that bacteriuria is preceded by infection of the urethra which in turn has followed colonization of the vaginal orifice by the causative organism. It would seem, therefore, both kind and prudent to treat all patients presenting with urinary tract symptoms associated with pyuria irrespective of whether they are subsequently proved to have bacteriuria.

All four partners in this country practice are so convinced of the value of urine microscopy that we have four microscopes, one in each branch surgery. To us it is surprising that it is so rarely used by either general practitioners or hospital clinicians.

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