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Differential Count and Quantitative Estimation of Granulocytes, Mononuclear Leukocytes and Renal Epithelial Cells in Urine

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ABSTRACT

Voided urine samples from healthy persons and patients with glomerulonephritis, chronic pyelonephritis and bacterial urinary tract infection were examined. Urine from healthy persons contained 0–12 granulocytes/mm^3, 0 mononuclear leukocytes/mm^3 and 0–2 renal epithelial cells/mm^3. Urine from patients contained a larger number of cells/mm^3 than did urine from healthy persons. With differential counting of granulocytes, mononuclear leukocytes and renal epithelial cells patients with glomerulonephritis could be separated from patients with chronic pyelonephritis or bacterial urinary tract infection. The percentage values obtained at differential counting were not correlated to age, sex, total number of cells/mm^3, proteinuria or serum creatinine level.

INTRODUCTION

It is well known, that the number of urinary leukocytes is generally increased in urinary tract infection (UTI), but often normal in chronic pyelonephritis without presence of infection (4, 6, 8). The percentages of urinary granulocytes (GRAN), mononuclear leukocytes (MONO) and renal epithelial cells (REP) vary between different diseases (7). A greater percentage of urinary GRAN in chronic pyelonephritis and UTI compared to glomerulonephritis (7) might be caused by an increase of GRAN/mm^3, while MONO and REP/mm^3 might remain unchanged. On the other hand, the possibility of an increase of MONO and REP/mm^3 in glomerulonephritis can not be excluded. The aim of the present investigation was to study what quantitative changes underlie the different percentage distribution of GRAN, MONO and REP in healthy and diseased persons.

MATERIAL

Healthy persons, Group I

30 persons were examined. 14 were males (median age 34 years, range 23–49) and 16 females (median age 26 years, range 19–56). All of them were working or studying at the hospital. They did not have any known disease. None of them had had any renal or urinary tract disease.

Patients, Groups II–IV

134 in-patients at the Department of Nephrology were examined. The serum creatinine level was determined. The degree of proteinuria was measured as grams of albumin per 24 hours in 40 patients. Group II contains patients with a clinical diagnosis of glomerulonephritis, lupus nephritis or Goodpasture's syndrome without UTI. Group III contains patients with chronic pyelonephritis. Group IV contains patients who had UTI, but no pyelonephritis.

Group II contains 32 patients. 18 were males and 14 females. Their median age was 31.5 years (range 15–72). Glomerulonephritis was confirmed with renal biopsy and renal angiography in 25 patients. Eight patients had lupus nephritis, one had Goodpasture's syndrome. The remaining patients had the following types of glomerulonephritis: acute ($n=5$), proliferative ($n=7$), membrano-proliferative ($n=2$), minimal change ($n=1$), membranous ($n=2$) and unclassified ($n=6$). No patient had UTI symptoms or significant bacteriuria ($\geq 10^5$ bacteria/ml of urine). The activity of the disease was very varying, some patients being subjectively healthy since several years, some rapidly deteriorating.

Group III contains 15 patients with a clinical diagnosis of pyelonephritis. Three were males and twelve females. Their median age was 45 years (range 22–68). Renal biopsy showed interstitial nephritis in seven patients. All patients had or had had UTI. Intravenous pyelography in all of them showed lesions interpreted as pyelonephritis. Bacterial urinary culture was performed in 13 patients, showing significant bacteriuria in three of them.

Group IV contains 18 patients with UTI but no pyelonephritis. Ten were males and eight females. Their median age was 56.5 years (range 18–80). All had symptoms clinically interpreted as UTI and/or significant bacteriuria during the last two months. Several patients had received anti-bacterial therapy prior to the examination. Urine culture in six out of ten patients showed significant bacteriuria at the time of examination. Patients in this group had renal carcinoma, renal amyloidosis, primary amyloidosis, nephrosclerosis, glomerulonephritis ($n=1$), bladder calculi, prostatic cancer, prostatic hyperplasia, hydronephrosis, nephronophtisis or nephrocalcinosis.

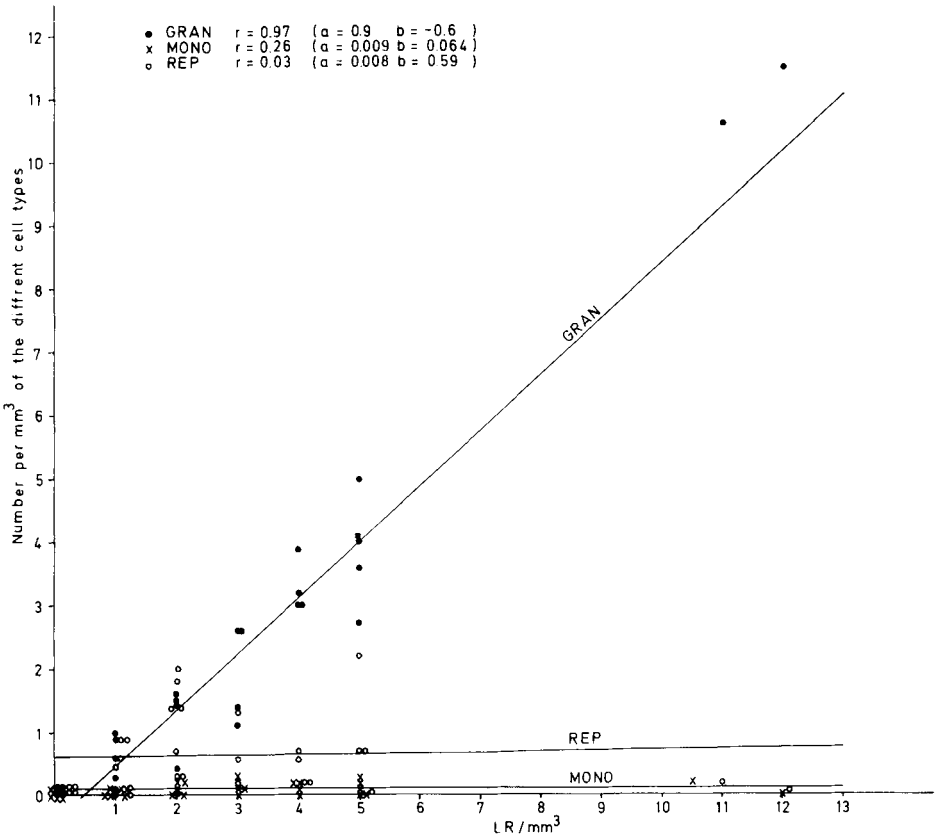

Fig. 1. The number of granulocytes varied, but the number of mononuclear leukocytes and renal epithelial cells was constant.

METHODS

Voided, non-morning, urine samples were used. Counting of cells/mm³ in uncentrifuged urine and examination of the corresponding wet sediments was performed by the author within one hour after sampling.

Counting of leukocytes plus REP/mm³ in uncentrifuged urine

30 samples from healthy persons and 155 samples from 134 patients were examined. After shaking and stirring the sample, a Bürker haemocytometer was filled and cell counting was performed at ×400 magnification. This technique as a rule did not permit accurate identification of different kinds of leukocytes or REP. Leukocytes and REP consequently were counted together and will be referred to as LR. Squamous epithelial cells and urothelial cells were excluded from the count. All squares in the chamber were searched or counting was stopped, when a minimum of 100 LR had been counted (3).

Examination of urinary wet sediment

10 ml of urine was centrifuged at 1 500 r.p.m. for 10 min. The supernatant was carefully poured off and the sediment was resuspended by vigorous shaking. A small drop was placed on a slide, coverglass was applied and a very thin film of sediment was obtained. The sediment was examined under a Zeiss phase contrast microscope. With use of immersion oil and ×1 000 magnification 100 cells were identified and the percentages of GRAN, MONO and REP were determined. The method has been described previously (7, 9).

Examination of fixed and stained sediment from healthy persons

As a small number of cells often leads to failure of the differential count an additional method was used in healthy persons. A 100 ml sample was used. After counting of LR/mm³ and examination of the wet sediment as described above, the rest of the sample was centrifuged at 3 000 r.p.m. for 15 min. The sediment was resuspended in 10 ml of physiological saline and recentrifuged at 3 000 r.p.m. for 15 min. A smear was prepared from the sediment. It was fixed, Papanicolaou-stained and mounted. Differential counting of GRAN, MONO and REP was performed, using previously described criteria (9).

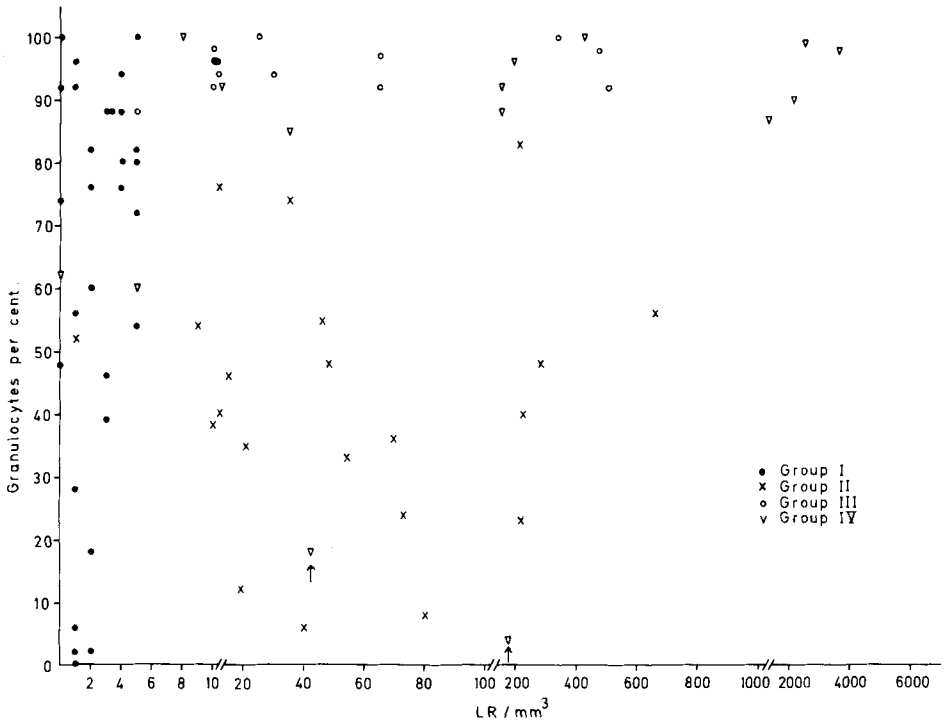

Fig. 2. A smaller number of cells/mm³ was found in Group I than in the patient groups. All patients in Groups III and IV had a great percentage of granulocytes, except two patients (arrows) who had hydronephrosis.

Statistical methods

As level of significance was chosen $p<0.05$. Correlation coefficients (r) were calculated according to the method of Pearson. Wilcoxon's rank sum test was also used applying a two-sided hypothesis.

RESULTS

Group I

The median number of LR/mm³ was 2.5 (range 0–12). Urine from females contained a larger number of LR/mm³ than males, but the difference was not statistically significant. The number of GRAN/mm³ (Fig. 1) was larger in urine from females (median 3/mm³) than males (median 0.5/mm³). The difference was statistically significant ($p<0.02$). Fig. 1 shows that an increase of GRAN/mm³ was correlated to an increase of LR/mm³. The median number of REP/mm³ was 0.5 (range 0–2.2). The number of MONO/mm³ was small, <0.5 in all samples. The number of MONO or REP/mm³ was not correlated to the number of LR/mm³ (Fig. 1). No sex difference was found with respect to number of MONO and REP/mm³. No influence of age was found.

Groups II–IV

The median number of LR was 20/mm³ in Group II, 14/mm³ in Group III and 39/mm³ in Group IV (Figs. 2–4). A differential count was obtained from 23 patients in Group II, 11 patients in Group III and 15 patients in Group IV. Median number of GRAN, MONO and REP/mm³ and median percentage of the three cell categories is shown in Table I. The distribution of cells is shown in Figs. 2–4.

Comparisons between groups

The number of LR/mm³ was larger in the three patient groups than in Group I ($p<0.05$). The percentage of GRAN was statistically significantly greater in Group III and Group IV than in Group II (Table I, Fig. 2). Two patients in Group IV, however, had a small percentage of GRAN, 4% and 18% respectively, and a great percentage on MONO (Fig. 3). These two patients had

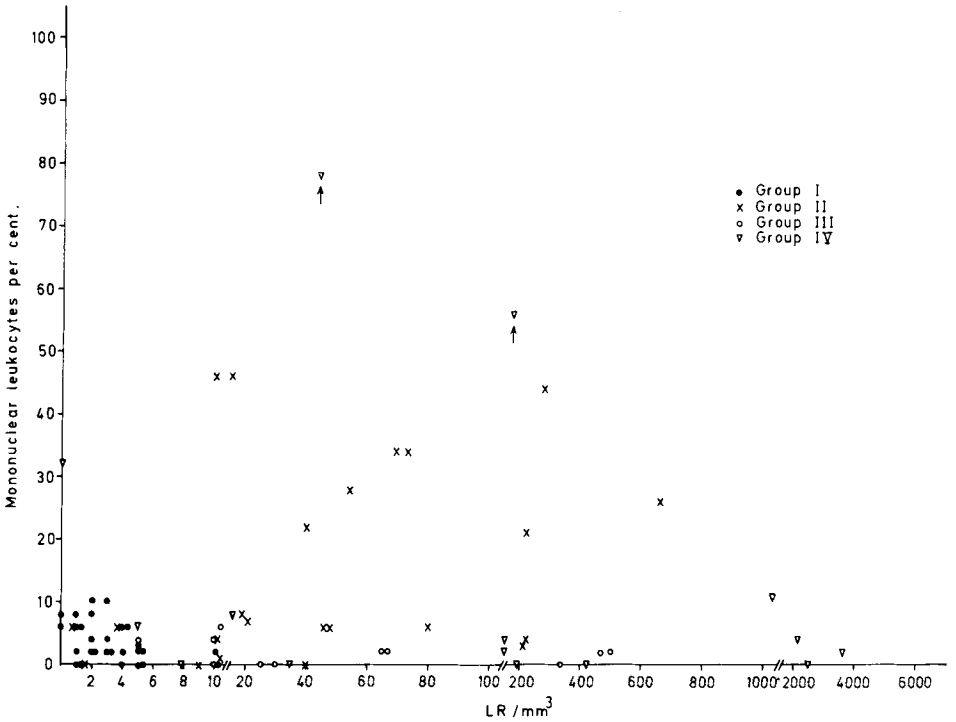

Fig. 3. More than 20% mononuclear leukocytes was found in nine patients belonging to Group II and three patients in Group IV. Two of these latter patients (arrows) had hydronephrosis.

hydronephrosis. A great percentage of MONO (histiocytes) may occur in this disease (10). The percentages of MONO and REP were greater in Group II than in Groups III and IV ($p<0.05$).

There was no statistically significant correlation between number of LR/mm³ and percentage or number/mm³ of any of the three cell categories in any of the patient groups ($r\leq0.34$). Nor was any statistically significant correlation found between albuminuria, measured as grams of albumin/24 h, or serum creatinine and percentage or number/mm³ of MONO and REP ($r\leq0.34$). Age or sex was not found to have any influence upon percentage or number/mm³ of GRAN, MONO or REP in any of the patient groups. No statistically significant difference in percentage or number/mm³ of GRAN, MONO and REP was obtained between Group II patients divided according to type of glomerulonephritis. It might be added, that Group II patients with clinically "inactive" disease had a small number of cells/mm³.

The influence of bacteriuria upon the number of LR/mm³ was studied in the following way: 23 patients in Groups III and IV were divided into two subgroups: nine patients with significant bacteriuria at the time of examination and 14 patients without. The number of LR/mm³ was larger in patients with bacteriuria (median 336/mm³) than without (median 22/mm³, $p<0.02$). The number of LR/mm³ in patients with chronic pyelonephritis but without significant bacteriuria in Group III was statistically significantly greater than the number of LR/mm³ in Group I.

DISCUSSION AND CONCLUSIONS

Most investigators have found ≤10 unspecified leukocytes/mm³ in "normal" urine (1, 11), some report higher figures (5). Gadeholt, with a technique similar to mine, counted leukocytes plus non-squamous epithelial cells in urine from 275 healthy males and females and found ≤13 cells/mm³ in 95% of them (2). Comparison of the distribution of LR/mm³ in the present healthy population to the figures for leukocytes plus non-squamous epithelial cells reported by Gadeholt (2) was performed with χ^2-analysis. No statistically significant difference was obtained ($p>0.05$). In the present material

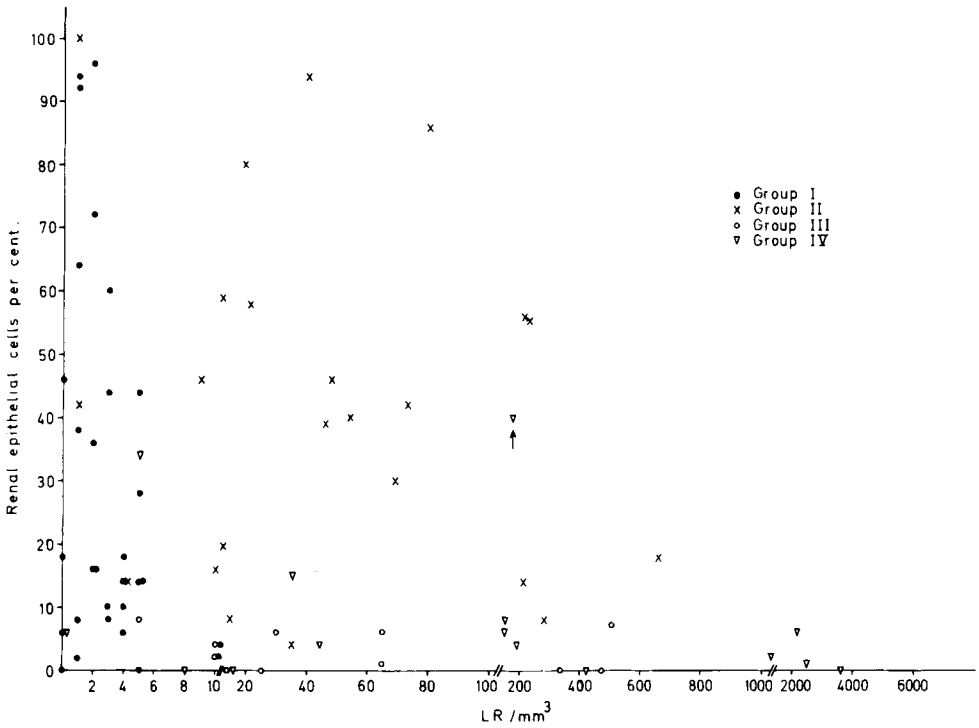

Fig. 4. A greater percentage of renal epithelial cells was found in Group II than in Groups III and IV. One Group IV patient (arrow) with cancer of the prostate and bilateral ureteral obstruction also had a large number of renal epithelial cells/mm³.

the number of granulocytes/mm³ was statistically significantly greater in urine from healthy females than healthy males. Also, the number of leukocytes/mm³ in Gadeholt's material was greater in urine from females than males. Thus, it might be inaccurate to use the same upper limit of normal number of cells/mm³ for both males and females. The varying number of GRAN/mm³ in urine from healthy persons might be caused by a varying degree of contamination, which could also explain the greater number of GRAN/mm³ in healthy females. The number of MONO and REP/mm³ was constant and small in urine from healthy males and females. It seems reasonable to assume that the sloughing off of REP from the kidney is rather constant in healthy persons. The variation in number of GRAN/mm³ explains the varying percentage found in urine from healthy persons.

The majority of the patients with UTI but no pyelonephritis had other kidney or urinary tract diseases known to be accompanied by a small percentage of granulocytes (7). It seems reasonable to assume that UTI itself might lead to an increased granulocyte excretion such as was also found in patients with chronic pyelonephritis even without concomitant bacteriuria. Mononuclear

Table I. *Median values of GRAN, MONO and REP*

Group	GRAN%	GRAN/mm³	MONO%	MONO/mm³	REP%	REP/mm³
I	78	1.5	2	0	16	0.5
II	40	9	6	3	42	18
III	94	28	2	0	2	0
IV	90	135	4	1	4	5

leukocytes and renal epithelial cells as a rule did not increase in UTI or chronic pyelonephritis. In glomerulonephritis an increased excretion of mononuclear leukocytes and renal epithelial cells was found, as well as granulocytes. It is possible, that mononuclear leukocytes and renal epithelial cells in glomerulonephritis might reflect the activity of the disease. The percentage distribution of the different cells did not vary with the total number of cells within the different patient groups. No influence was found of sex, age, proteinuria or serum creatinine level upon the differential count.

Estimation of the number of cells/mm^3 thus seems important for discrimination between healthy and diseased persons and perhaps also for assessment of disease activity. Differential counting of cells on the other hand might be of value for discrimination between different diseases. Optimal information is obtained through the combined use of the two methods.

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