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COMPARISON OF URINE CYTOLOGY VERSUS FINE NEEDLE ASPIRATION CYTOLOGY IN MONITORING RENAL ALLOGRAFT DYSFUNCTION

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Summary

In renal allograft monitoring we performed daily urine cytology (UC) (n = 773) and fine needle aspiration cytology (FNAC) (n = 144) two to three times weekly in 29 allograft recipients with 18 acute rejection episodes. UC is more accurate in diagnosis and therapeutic control of acute rejection episodes. In addition UC is better than FNAC in revealing non-rejection causes of graft dysfunction such as interstitial nephritis (pyelonephritis, allergic nephritis), the presence of bacteria, fungi and virus inclusion bearing cells in the sediment and nephrotoxic tubular damage.

Introduction

Urinary cytological (UC) parameters used for diagnosis of renal allograft rejection in the past have been increased excretion of lymphocytes [1], excretion of more than 25,000 lymphocytes/hour [2] or 50,000 lymphocytes/hour [3] and urinary excretion of pyronine positive lymphocytes [4]. Taft and Flax [5] demonstrated increased numbers of renal tubular cells in the urine. Bossen et al [6] described a urinary cytological profile during rejection consisting of background debris, lymphocytes, renal tubular cells, mixed cell clusters and casts. During acute rejection episodes, however, no particular cell type diagnostic of rejection could be identified by these authors. Schumann et al [7] found the renal epithelial cells from collecting ducts of greatest value in diagnosing an acute rejection episode [8] and presented a semiquantitative method of preparing urinary specimens for cytology using the cytocentrifuge. Pasternack et al [9] suggested assessing the in situ infiltrate found in rejection by means of repeated fine needle aspiration biopsy (FNAC) of the transplant. They found a good correlation between the presence of lymphoblasts in aspirates and the clinical evidence of rejection. Häyry et al [10] refined the FNAC method by comparing the aspirate with blood, calculating a differential. Because of the apparently different

diagnostic value of its cellular components they introduced the 'corrected increment' [11].

The purpose of this study was to determine which of these tests is most effective in monitoring transplant recipients for rejection.

Methods

From July 1981 to July 1982 we performed in 29 allograft recipients daily urine cytology (n = 773) and FNAC (n = 144) two to three times weekly.

Preparation of the UC specimens [7]

1. Ten millilitres of morning urine is spun in a standard centrifuge at 1500RPM for 10 minutes.
2. Sediment is resuspended in one millilitre supernatant.
3. Two slides using two to six drops (depending on the sediment button) of the resuspended specimen are spun in a cytocentrifuge (Shandon Labortechnik, Frankfurt) at 750RPM for two to six minutes.
4. One to two drops of parlodion (one gram nitrocellulose in 200mg 95% ethanol and 200ml anhydrous ether) are applied to the cellular area.
5. The slides are fixed in 95 per cent ethanol.
6. Papanicolaou staining.
7. Both slides are screened noting background pattern, cellularity, erythrocytes, viral inclusions and abnormal cells. Ten high power fields are counted in the most cellular slide for neutrophils, lymphocytes, collecting duct cells, proximal and distal tubular cells, casts and fragments. The result is multiplied by six/ number of spun drops of resuspended sediment. More than 20 collecting duct cells or more than 20 lymphocytes, representing >one per cent of inflammatory cells on consecutive days were considered as evidence of rejection.

Preparation of the FNAC-specimens [10]

1. Insertion of a 0.7 × 90mm spinal needle (Terumo, Tokyo, Japan) percutaneously to the kidney transplant without anaesthesia.
2. Approximately 10–15μl of kidney cellular contents are drawn into 2.5ml of phosphate-buffered saline.
3. Approximately 200μl of the aspirate containing buffer are spun in a cytocentrifuge at 350RPM for seven mintues.
4. Fixation of the slides in methanol.
5. Preparation of a peripheral blood smear.
6. The slides are stained with May-Grünwald-Giemsa.
7. Differential count of all nucleated cells in the aspirate and blood.

The diagnosis and classification of transplant rejection is based on an increase of lymphoblasts, plasmablasts, plasmacells, lymphocytes, monocytes and macrophages in the biopsy specimen compared with the same cells in the peripheral blood (increment) [10]. Calculation of the corrected increment [11].

Clinical rejection criteria included fever, reduction of urinary output, graft tenderness, hypertension and an increase of >0.3mg% in serum creatinine.

Results

A summary of our FNAC and UC results in 29 allograft recipients with 18 clinical acute rejection episodes is presented in Table I. None of the allografts were lost due to treatment-resistant rejection. Base-line values of renal tubular cells in UC were established a few days after urine production of the transplant started Base-line values for increment were established during the first post-operative week.

In UC the diagnosis of acute rejection (AR) could be made one to three days before or on the day of the event, in two cases shortly after. The FNAC-increment usually was detectable one to two days earlier than findings in UC. In 4/18

TABLE I. Summary of fine needle aspiration biopsy and urine cytology findings in 29 allograft recipients

	FNAC (n = 144)	UC (n = 773)
Diagnosis of acute rejection predicted or confirmed	14/18 = 77.8%	18/18 = 100% Collecting duct cells in 18/18 Collecting duct cells plus lymphocytes in 10/18
False negative acute rejection diagnosis	4/18 = 22.2%	0/18 = 0%
False positive acute rejection diagnosis a) increment b) corrected increment	 19/144 specimens = 13.2% 18/144 specimens = 12.5%	Five episodes of isolated lymphocyturia in four patients
Specimen unsatisfactory	17/144 = 11.8%	11/773 = 1.4%
Complications	In one case intrarenal haematoma plus macrohaematuria Macrohaematuria of short duration in 4.9%	∅

(22.2%) of rejection episodes a diagnostic increment of lymphoblasts could be found, in 55.6 per cent the FNAC increment consisted of monocytes, lymphocytes and in few cases moderate to marked amounts of macrophages. In 22 per cent FNAC failed to show acute rejection. In 13.2 per cent or 12.5 per cent (calculation of corrected increment) the result was falsely positive. In 11.8 per cent the specimens contained no graft material.

Macrohaematuria of short duration was observed in 4.9 per cent, one patient developed an intrarenal haematoma (1.5 × 1.5cm).

UC corresponded in all cases with clinical evidence of AR. In 18/18 acute rejection episodes collecting duct cells, in 10/18 collecting duct cells accompanied by lymphocytes were diagnostic. In five cases of (isolated) lymphocyturia a false positive diagnosis of rejection was made. Concomitant cytologic findings in these episodes included excessive neutrophils in the urine, macrohaematuria, acute tubular necrosis (ATN) and chronic urinary tract infection. During successful antirejection therapy collecting duct cells and lymphocytes decreased to <20 per 10 high power fields. Rejections with appearance of increased lymphocytes responded better to treatment than cases with collecting duct cells and fragments (AR and ischaemic necrosis).

In 4/18 (22.2%) FNAC the increment remained abnormal despite successful treatment of rejection. Increments, indicating marked to irreversible rejections (15/144 = 10.4%) were seen in an otherwise benign clinical course.

UC in allograft dysfunction due to ATN or interstitial nephritis (pyelonephritis, allergic type), showed patterns clearly distinguishable from AR. Additional findings in the sediment have been tubular damage due to aminoglycosides (marked exfoliation of proximal tubular cells), crystalluria and urinary tract infections due to bacteria, fungi and virus (multinucleation and groundglass nuclear changes in herpes simplex infection). Besides assessment of the increment FNAC gave evidence for ATN showing degenerative changes in aspirated tubular cells.

Discussion

Comparison of the methods show that UC is more accurate than FNAC in the diagnosis and therapeutic control of an acute rejection episode, thereby contributing to more efficient treatment. The introduction of the corrected increment [11] did not increase the diagnostic accuracy of FNAC, which often gave false positive results in ATN, pyelonephritis and clinical evidence of virus infection. The presence of lymphoblasts [9] in our aspirates showed no good correlation with clinical rejection episodes.

In cases of macrohaematuria, marked neutrophiluria, chronic urinary tract infection, virus infection and ATN isolated lymphocyturia in UC may give a misleading diagnosis of AR. We agree therefore with Schumann [7, 8] that the appearance of renal tubular cells is more reliable than the appearance of lymphocytes in the diagnosis of an acute rejection episode. In addition to the excellent correlation of a rise in collecting duct cells with AR, UC gives more information regarding other causes of transplant dysfunction e.g. infections, allergic nephritis, nephrotoxic tubular damage.

An essential advantage of UC is its non-invasive nature, allowing daily monitoring without inconvenience to the patient.

References

1 Kaufmann HM, Clark RF, Magee JH et al. *Surg Gynecol Obstet 1964; 119:* 25
2 Spencer ES, Peterson VP. *Acta Med Scand 1967; 182:* 73
3 Papadimitriou M, Chisholm GD, Kulatilake AE et al. *J Clin Path 1970; 23:* 99
4 Hrushesky W, Sampson D, Murphy GP. *Arch Surg 1972; 105:* 424
5 Taft PD, Flax MH. *Transplantation 1966; 4:* 194
6 Bossen EH, Johnston WW, Amatulli J et al. *Acta Cytol 1970; 14:* 176
7 Schumann GB, Burleson RL, Henry JB et al. *Am J Clin Pathol 1977; 67 (2):* 134
8 Schumann GB, Weiss MA. In Schumann GB, Weiss MA, eds. *Atlas of Renal and Urinary Cytology and its Histopathologic Bases.* Philadelphia, Toronto: J B Lippincott Co
9 Pasternack A, Virolainen M, Häyry P. *J Urol 1973; 109:* 167
10 Häyry P, v Willebrand E, Ahonen J et al. *Transpl Proc 1980; 12(2):* 331
11 Häyry P, v Willebrand E, Ahonen J et al. *Ann Clin Res 1981; 13:* 264

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