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Pancreatic juice cytology for monitoring pancreatic grafts in the early postoperative period

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Abstract. Thirty-one pancreas transplant recipients were monitored by pancreatic juice cytology in the early postoperative period. An increase in the total amount of cells and, in particular, signs of immunoactivation with the appearance of two or more blast-transformed cells per specimen were taken as evidence of acute rejection. According to these criteria a total of 38 rejection episodes were diagnosed. The first positive cytology appeared after 9 days (mean) and lasted for 2 days (mean). Immunocytochemical analysis of the juice showed increased amounts of CD3 + cells during rejection. When rejection occurred during prophylaxis with antithymocyte globulin, neutrophils were preponderant in the pancreatic juice while during OKT-3 prophylaxis a high percentage of monocytes was a characteristic finding. Antirejection treatment was started when the cytology became positive and all rejection episodes except one were reversed. A decrease in the pancreatic juice amylase activity occurred in 66% of the rejection episodes, but in only 5 of the 38 episodes was the decrease highly significant. No correlation was found between graft rejection and volume excretion of pancreatic juice. There were no persistent or characteristic changes in serum amylase or peripheral white blood cell count at the time of rejection. Graft pancreatitis was diagnosed cytologically in 7 patients, in 5 of whom the grafts were eventually lost.

Key words: Rejection, pancreas transplantation, juice cytology – Cytology, pancreatic juice, rejection – Pancreas transplantation, juice cytology

Prompted by the report by Steiner et al. [15] of the appearance of an increased number of lymphocytes in the pancreatic juice during a renal graft rejection episode in a recipient of renal and pancreatic grafts, we developed a procedure for cytological evaluation of pancreatic juice in pancreatic transplant patients [14, 18]. Early experience with the method indicated that evidence of cellular immunoactivation in pancreatic juice is an earlier marker for pancreatic graft rejection episodes than are signs of exocrine dysfunction [18, 19]. Recently, we were able to demonstrate that, in comparison to the findings in graft histology, the cytological signs of rejection appear at an early stage. Furthermore, signs of graft pancreatitis could be detected by cytology [9]. The method has now become our main tool for the diagnosis of pancreatic graft rejection during the early postoperative period.

In the current series of patients acute pancreatic rejection was diagnosed by cytology according to our established criteria [14]. The aims of the present study were to evaluate the impact of different prophylactic regimens on the cellular composition in the juice, to clarify whether the introduction of immunocytochemistry for analysis of CD3 + cells in the juice would add relevant information, and to assess some other markers during rejection diagnosed by cytology. In addition, the findings in pancreatic juice during graft pancreatitis were evaluated and, finally, some experience concerning microorganisms in the juice was gained.

Materials and methods

Patients

Thirty-one patients suffering from type-1 diabetes and undergoing pancreatic transplantation between July 1987 and August 1990 were included in the study. There were 15 female and 16 male patients with a mean age of 34.3 years (range 18–47 years). Nineteen patients underwent simultaneous pancreatic and renal transplantation. Ten patients with preuremic diabetic nephropathy received a pancreatic graft only, and two received a pancreatic graft after a previous renal transplantation. The pancreatic graft 1-year survival rate among patients receiving simultaneous pancreatic and kidney grafts was 47% (2 out of 19 patients died during the 1st postoperative year); among patients receiving pancreatic grafts only it was 27% (2 out of 12 patients died during the 1st year).

Surgical procedure

All grafts were from cadaveric donors. Twenty-three patients received pancreaticoduodenal grafts while eight received segmental pancreatic grafts. The arterial pedicle of the graft was anastomosed

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to the recipient's right common iliac artery and the graft portal vein was connected to the inferior caval vein. Exocrine diversion was accomplished by enteroenterostomy between the graft duodenal segment and the recipient jejunum when pancreaticoduodenal grafts were used. When segmental grafts were used, a pancreaticoenterostomy was constituted. For the first few weeks after transplantation (mean 27 days, range 7–55 days), external pancreatic juice diversion was obtained by means of a pancreatic duct catheter passed through the enteric wall and further through the abdominal wall [6].

Immunosuppression

The immunosuppressive protocol consisted of cyclosporine, azathioprine and prednisolone. In 21 patients, rabbit polyclonal human T-lymphocyte antibody (ATG; Fresenius, FRG) 3 mg/kg was included in the prophylactic treatment protocol for the first 3–7 days (mean 6.6 days). Five patients received prophylactic treatment with murine monoclonal CD3 antibody (OKT-3, Ortho, USA) 5 mg/day for the first 4–11 days (mean 8.2 days). In these patients cyclosporine was withheld until the 7th postoperative day. Pancreatic graft rejection episodes were treated with intravenous bolus doses of methylprednisolone (1.25 g over 4 days) and if this was not effective OKT-3 was given for 5–7 days.

Pancreatic juice cytology

Samples for pancreatic juice cytology were collected once daily according to the method previously described in detail [14]. Briefly, three drops of pancreatic juice are collected in a centrifuge tube containing 5 ml tissue culture medium and the cells are centrifuged onto glass slides using a cytocentrifuge. After air drying, the specimens are stained according to a May-Grünwald-Giemsa procedure and examined by light microscopy. A total of 778 specimens were analyzed. Routinely, the total amount of cells, the relative contribution of mononuclear cells, and the occurrence of blast-transformed cells are recorded.

For the purpose of this study, the relative contribution of different types of inflammatory cells, i.e. blast-transformed cells, lymphocytes, monocytes, and macrophages, were evaluated during 38 rejection episodes by differential counting of 200 cells. Due to suboptimal cell morphology, some cells in most specimens could not be classified unequivocally. Those cells were termed ghost cells and recorded separately. The total amount of cells in each specimen, expressed as cell density, was estimated by means of an eyepiece-integrated point counter (Zeiss Integralplatte 100/25) from 20 fields of vision at high magnification.

From January 1989, conventional cytology was supplemented with an immunocytochemical analysis. The specimens were prepared as for conventional pancreatic juice cytology but with the following modifications. After air drying, the specimens were fixed in acetone for 10 min at 4 °C. After rinsing with TRIS-saline buffer (pH 7.6), the specimens were incubated with a primary antibody [anti-Leu 4 (CD3), Becton & Dickinson AB, Sweden] for 30 min at room temperature. The specimens were then rinsed and immersed in TRIS-saline buffer for 10 min at room temperature. Alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Sweden) was used as a secondary antibody (30 min at room temperature), and alkaline-phosphatase-conjugated swine anti-rabbit immunoglobulin (Dakopatts, Sweden) was used as a tertiary antibody. Finally, the specimens were stained with fast red, followed by hematoxylin. Light microscopic examination was performed in a total of 199 immunocytochemical specimens; the cell density and the density of CD3 + cells in the specimens were estimated by point counting as above.

Cytological diagnosis of acute graft rejection. Rejection was diagnosed when a specimen was cell-rich with a large proportion of mononuclear cells and contained at least two blast-transformed cells.

Cytological diagnosis of graft pancreatitis. Graft pancreatitis was considered to be present when a cytological specimen became cell-rich following a period of low cellularity, and contained larger numbers of neutrophils, ghost cells, monocytes, epithelial cells, and macrophages. Necrotic tissue fragments were present in some but not all instances.

Diagnosis of renal graft rejection episodes

Renal graft rejection episodes were diagnosed by an increase in serum creatinine that could not be explained otherwise and/or by the histological or cytological findings in core needle biopsies and fine-needle aspiration biopsies, respectively.

Amylase activity in the pancreatic juice and serum

Amylase activity in the pancreatic juice was measured twice daily and in the serum once daily, using the catalytic method of Rausher et al. [13].

Statistics

Figures are given as means and standard deviation (SD). The χ^2 test or Kruskal-Wallis test were used for the statistical analysis.

Results

Acute rejection episodes

A total of 38 pancreatic graft rejection episodes were diagnosed by means of pancreatic juice cytology (mean 1.2 rejection episodes per patient). Seven patients suffered no rejection episode, one episode was diagnosed in 12, two episodes in 10, and three episodes in 2 patients. Among patients with simultaneous pancreatic and renal transplants, 21 renal graft rejection episodes were diagnosed. In 16 of these episodes, pancreatic graft rejection as diagnosed by pancreatic juice cytology occurred concomitantly. The mean interval between transplantation and the first episode of positive cytology was 9 days (range 3–22 days, $n = 21$). Six of the rejection episodes occurred during prophylactic treatment with ATG or OKT-3. Eighty-four specimens showed cytological findings characteristic of rejection (mean 2.2 positive specimens per episode, range 1–5 positive specimens). Antirejection treatment was given for a total of 150 days (mean 3.9 days per episode, range 0–10 days). Following antirejection treatment all rejection episodes except one were reversed insofar as juice cytology reverted to normal. In one patient the antirejection treatment was discontinued because of the patient's generally deteriorating clinical condition, and 2 days later the graft was removed. Subsequent histological examination of the surgical specimen showed rejection.

Pancreatic juice cytology during rejection. As reported previously, the pancreatic juice always contains a high number of cells during the first 3–5 days after transplantation [14]. The juice then usually becomes essentially void

Table 1. Cell density and cell composition of specimens obtained during acute rejection episodes

	During triple therapy and ATG ($n = 3$)		During triple therapy and OKT3 ($n = 3$)		During triple therapy ($n = 32$)	
	Mean	SD	Mean	SD	Mean	SD
Cell density	304.3	295.4	131.3	44.9	205.6	180.5
Lymphoblasts	0.1	0.1	0		0.5	0.6
Activated lymphocytes	0.1	0.1	0.2	0.2	1.2	1.9
Lymphocytes	0.2	0.1	0.3	0.4	2.1	3.1
Neutrophils	83.9	4.0	39.7	2.0	69.1	19.6
Eosinophils	0.8	1.0	2.5	1.2	4.2	9.5
Monoblasts	0.5	0.4	0.2	0.2	0.2	0.2
Monocytes	12.4	3.8	49.3	3.6	19.0	12.2
Macrophages	0.5	0.7	0		0.3	0.7
Ghost cells	1.5	0.7	8.0	1.8	3.6	3.2
Epithelial cells[a]	0.8	0.5	0.8	0.2	1.6	1.9

[a] The percentage of epithelial cells was estimated against the number of the other cells

of cells, but in the case of an acute rejection episode (or pancreatitis) the cellularity increases again. The findings in the pancreatic juice cytology during rejection episodes occurring during prophylactic treatment with ATG or OKT-3, or during rejection episodes occurring when these drugs had been discontinued and the patients were on triple drug regimen only, have been analyzed separately (Table 1). When rejection occurred during triple drug therapy only ($n = 32$), cellular density was 206 (181). Characteristic findings were a high percentage of neutrophils (69%) and monocytes (19%). Lymphoblasts, activated lymphocytes, lymphocytes, eosinophils, and monoblasts each constituted less than 5%. Unclassifiable ghost cells amounted to 4% and an occasional epithelial cell was also seen. Whether the patients had been receiving prophylactic ATG or OKT-3 in the early postoperative period before the rejection made no difference. Rejection episodes occurring during prophylactic ATG treatment were characterized by a somewhat higher percentage of neutrophils (84%) and a lower percentage of monocytes (12%) than those found in rejection occurring during triple drug therapy. Rejection occurring during prophylactic OKT-3 administration also followed a different pattern of juice cytology: the percentage of neutrophils was lower than in rejections occurring under the triple drug regimen (40%), while the percentage of monocytes was higher (49%). In both groups of patients lymphocytes were essentially absent from the juice during rejection episodes (Table 1).

CD3 + cells in pancreatic juice during rejection. For the first few days after the transplantation, when the cellular density was high, no CD3 + cells were observed except in one patient. During periods of stable graft function, CD3 + cells were never observed in the pancreatic juice. CD3 + cells were looked for during 11 rejection episodes and found in all instances except one (Fig. 1). Three of the rejections occurred during prophylactic OKT-3 treatment and eight occurred when only triple drug therapy was being given. Following treatment for rejection, the CD3 + cells disappeared. When the transplanted kidney suffered acute rejection, but the pancreatic graft was unaffected, a slight increase in the number of CD3 + cells was observed in the pancreatic juice in some instances. The presence of bacteria in the pancreatic juice was not accompanied by an increase in CD3 + cell count.

Pancreatic juice volume flow during rejection. The volume of pancreatic juice produced per 24 h before rejection episodes, during rejection episodes, and after antirejection treatment was 754 ± 422, 775 ± 436, and 878 ± 452 ml, respectively. A significant decrease was found in 8 rejection episodes, while an increase was seen in 16. In 14 instances there was no change.

Pancreatic juice amylase activity during rejection. The mean pancreatic juice amylase activity before rejection episodes was 5009 ± 3334 μkat/l. The lowest value during rejection episodes was 2445 ± 2202 μkat/l and after antirejection treatment 6118 ± 3906 μkat/l (Fig. 2). The pan-

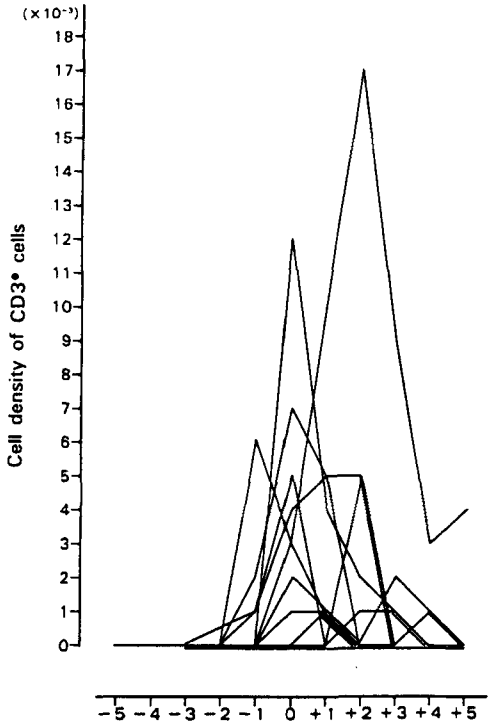

Fig. 1. Density of CD3 + cells in the pancreatic juice during pancreatic graft rejection episodes

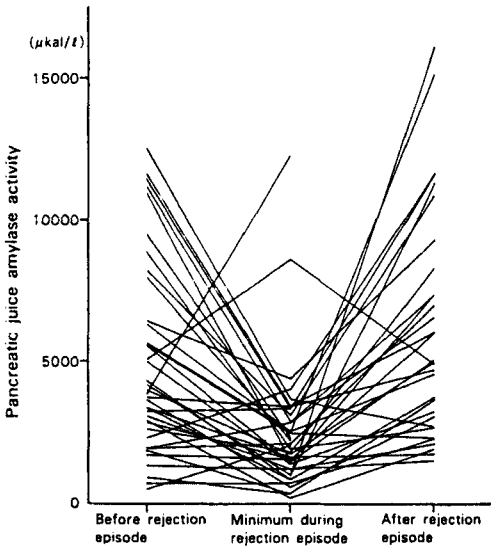

Fig. 2. Pancreatic juice amylase activity before, during, and after 38 pancreatic graft rejection episodes

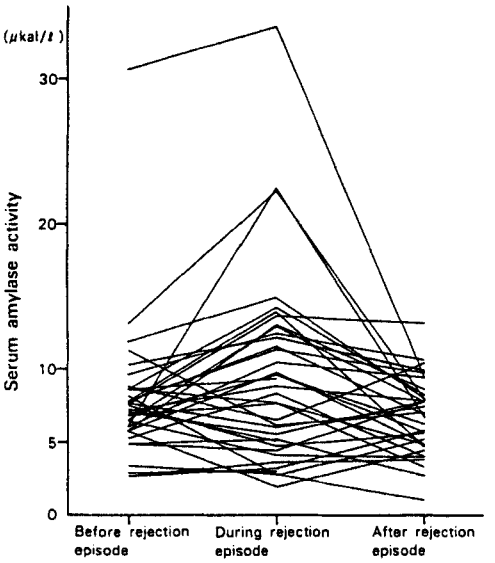

Fig. 3. Serum amylase activity before, during, and after 38 pancreatic graft rejection episodes

creatic juice amylase activity was unchanged during nine rejection episodes (continuously low in three), and increased during four. It was decreased during 25 episodes, the lowest level being above 2000 μkat/l in 11 episodes, between 1000 and 2000 μkat/l in 9 episodes and less than 1000 μkat/l in 5. After antirejection treatment the pancreatic juice amylase activity increased except in four cases (decreased in two instances, unchanged in two).

Serum amylase activity during rejection. Serum amylase activity before rejection episodes, during rejection episodes, and after antirejection treatment was 7.7 ± 4.4, 9.5 ± 6.4, and 6.7 ± 2.5 μkat/l, respectively (Fig. 3). An increase in the serum amylase activity was observed in 18 rejection episodes, whereas a decrease was seen in 9. There was no change in 11 instances.

White blood cell count during rejection. The figures before rejection episodes, during rejection episodes, and after antirejection treatment were 10.4 ± 3.8, 11.5 ± 4.6, and $11.0 \pm 4.3 \times 10^9$/l, respectively. An increase in the number of white blood cells was found in 16 rejection episodes, while a decrease was seen in 9. In 13 instances there was no change.

Cytological findings in pancreatic juice during pancreatitis

In 17 specimens obtained from seven patients, cytological signs of pancreatitis were observed. Five grafts were later removed and histological examination demonstrated pancreatitis in four and chronic rejection in one. Two grafts recovered after the disappearance of the cytological signs of pancreatitits.

Bacteria or fungi in the pancreatic juice

In 87 specimens (11.2%) obtained from 20 patients, bacteria or fungi were observed. Seventy-nine specimens contained bacteria, seven contained fungi, and one specimen contained both. There was no difference in the incidence of microorganisms between patients receiving prophylactic ATG and those treated with OKT-3. In ten instances, the microorganisms appeared after antirejection treatment. The microorganisms disappeared with time in six cases. In three patients the catheter was removed and in one patient the pancreas graft was removed because of an abscess around the graft. In another eight patients bacteria or fungi were observed when the intraductal catheter had been in place for a prolonged time period (mean 28.1 days, range 19–39 days). The catheter was removed in seven patients and in one patient the pancreatic graft was excised because of cytomegaloviral pancreatitis. In one patient fungi were observed on days 9 and 10, and the graft was removed on day 10. The histology showed fungal pancreatitis.

Discussion

Today, pancreatic juice cytology and amylase activity are the main markers of pancreatic graft rejection episodes. Our initial experience with pancreatic juice cytology demonstrated that cytological signs of acute rejection may precede a decrease in the pancreatic juice amylase level. Another important observation was that cytology occasionally was negative in spite of a significant decrease in pancreatic juice amylase activity [17, 19, 20]. Earlier, such episodes were treated by us as rejection episodes. With increased experience, however, signs of immunoactivation in the pancreatic juice cytology became our main diagnostic criterion for rejection. We then found that recovery was possible without antirejection treatment in cases where the amylase activity in the juice had decreased while the cytology remained negative [20]. Thus, pancreatic juice amylase is a less specific marker of rejection and

there is a risk of false-positive diagnosis of rejection if only amylase is studied [2, 8, 20].

The findings in the present study confirm and extend our previous observations. When positive juice cytology was taken as the criterion for rejection, the pancreatic juice amylase activity was found to be unchanged or increased in 13 out of 38 rejection episodes. In the remaining 25 episodes a decrease was observed but in 11 of these, the activity remained above 2000 μkat/l, a level which we consider normal [16]. Thus, in 24 instances (63 %) the pancreatic juice amylase level did not sink below the normal lower limit. The explanation for this probably lies in the fact that the diagnosis was made and treatment started early during the rejection episode in these cases, before exocrine dysfunction had developed.

When combined kidney and pancreas transplantation is performed, renal graft rejection is usually taken as a marker of pancreatic graft rejection as well [3, 4, 16]. In the present series, 21 renal graft rejection episodes were diagnosed, but only 16 of them were accompanied by pancreatic graft rejection. In some instances the antirejection treatment given for the renal graft rejection may have prevented or masked pancreatic graft rejection. Still, this finding suggests that rejection may occur independently in the two organs [11].

When rejection episodes occurred during triple drug therapy, a high proportion of neutrophils and monocytes appeared in the pancreatic juice. Interestingly, there were some differences in the cellular pattern if the rejection occurred during prophylactic ATG or OKT-3 treatment. In both instances the number of lymphocytes and activated cells was markedly suppressed as could be expected. The number of monocytes was particularly low when rejection occurred during ATG treatment; in contrast, it was relatively high when it occurred during OKT-3 treatment. The number of cases studied was small, however, and the apparent differences did not reach statistical significance. Further studies are required to confirm these findings and to asses their potential importance. Somewhat disappointingly, six rejection episodes were seen during ongoing prophylactic ATG or OKT-3 therapy.

Immunocytochemical analysis of the pancreatic juice using a monoclonal antibody against CD3 + cells appears to be a useful additional measure: during acute rejection, including that occurring during prophylactic OKT-3 treatment, the number of CD-3 + cells increased. This hallmark of immunoactivation may be a very specific herald of rejection. An increased mononuclear cell count has been observed when bacteria or fungi appear in the pancreatic juice [7]. However, no increase in the number of CD3 + cells was observed in the presence of bacteria or fungi.

Graft pancreatitis is a rather common complication after transplantation. The diagnosis is usually based on symptoms and signs such as local pain, fever, high serum amylase activity, and high white blood cell count [10, 12]. In our series, graft pancreatitis was indicated by cytology in seven patients, and four of these grafts did indeed have to be removed because of pancreatitis. Thus, cytological signs of pancreatitis seem to carry a bad prognosis. Although the diagnostic role of juice cytology for graft pancreatitis is not yet fully established, we believe that the method is helpful for the diagnosis [9], as has also been reported by others [7].

The presence of bacteria or fungi in the juice was usually not related to clinical signs of infection. Particularly, after antirejection treatment, bacteria or fungi were frequently observed. The use of OKT-3 may be a contributing factor [1, 5]; prolonged use of an intraductal catheter may be another causative factor of the appearance of bacteria or fungi in the juice [7]. An attractive feature of pancreatic juice cytology is that positive findings reflect the immunological process in the graft, while chemical parameters such as juice amylase activity reflect the tissue injury that occurs as a consequence of the immunological attack. Such a quick and sensitive marker of rejection might be used for individual tailoring of antirejection treatment. Indeed, we have recently limited or expanded the duration of treatment according to the cytological findings.

The method requires access to the pancreatic juice. This can be accomplished by introducing a temporary pancreatic duct catheter which is brought to the exterior – such catheters have been used by us since 1981 [6] with few, if any, complications. Long-term use of a ductal catheter is, however, impractical and probably harmful. Anyhow, pancreatic graft injury due to rejection or pancreatitis most commonly occurs in the early postoperative period when the catheter is still in place.

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