# Increased serum concentration of carbohydrate-deficient transferrin in patients with combined pancreas and kidney transplantation

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**Serum concentration of carbohydrate-deficient transferrin (cCDT) is used for laboratory diagnosis and follow-up of chronic alcohol abuse. In analyzing by CDTect-RIA (Pharmacia) sera from outpatients with combined pancreas and kidney transplantation and no excessive alcohol consumption, we found above-normal values for cCDT and CDT/transferrin ratios (CDT/Tf) in more than half of the samples. Isoelectric focusing of these samples showed distinct bands of carbohydrate-deficient isotransferrins, supporting the abnormal findings from the CDTect assay. In contrast, diabetics and outpatients who had received only kidney transplants showed normal values for cCDT, CDT/Tf, and isotransferrin patterns. Increased serum Tf, sialidase-producing microorganisms, and immunosuppressive medication were eliminated as causes of these abnormal cCDT and CDT/Tf results. Successful pancreas transplantation leads to hyperinsulinemia and normoglycemia, in contrast to hypoinsulinemia and hyperglycemia in the patients who receive kidney transplants alone. These factors may have pathogenic importance for CDT increase, yielding results falsely interpreted as positive with respect to alcohol abuse in patients with combined pancreas and kidney transplantation.**

**INDEXING TERMS:** alcoholism • isoelectric focusing • isotransferrins • sialic acid-deficient transferrins

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Isoelectric focusing (IEF) demonstrates a distinct microheterogeneity of human serum transferrin (Tf).[4] This phenomenon is the result of (*a*) altered protein moieties (genetic variants, e.g., Tf-C, Tf-D, and Tf-B), (*b*) variations in iron load (no iron bound to Tf: $Fe_0$-Tfs; iron bound to the C-terminal or N-terminal binding site: $Fe_{1C}$- or $Fe_{1N}$-Tfs; both binding sites loaded with iron: $Fe_2$-Tfs), and (*c*) different carbohydrate chains, with 0 to 8 sialic acid residues (asialo-, monosialo-, . . . , octasialo-Tfs) [1–3]. To reduce the number of Tf bands in IEF, analysis is often done after in vitro saturation of Tf with $Fe^{3+}$, yielding only $Fe_2$-Tfs (no $Fe_0$- and $Fe_1$-Tfs) [4–6]. In serum given this pretreatment, the main isotransferrin in Caucasians is tetrasialo-$Fe_2$-Tf (70–80%), phenotype Tf-C, with pI 5.4 [1–3].

Increased concentrations of sialic acid-deficient $Fe_2$-isotransferrins (a-, mono-, and disialo-$Fe_2$-Tf) with pI values $\geq 5.7$ are detected in serum of alcoholics [7]. Analysis of these isotransferrins, collectively referred to as carbohydrate-deficient transferrin (CDT), is widely used for detection and follow-up of chronic alcohol abuse [7–15].

In contrast to earlier reports [7, 16–18], several recently published studies [9, 10, 15, 19–21] indicate a diagnostic sensitivity of CDT that is not superior to those of other commonly used markers of alcohol abuse, e.g., γ-glutamyltransferase (γ-GT; EC 2.3.2.2) and mean corpuscular volume of erythrocytes (MCV), especially in female populations. In comparison with γ-GT and MCV, however, CDT shows the overall best diagnostic specificity: >90% [7, 9, 10]. Some cases of increased cCDT in the absence of alcohol abuse have been reported in patients with heart,

pancreas, or lung disorders, malignancy, or hypertension [13, 19, 20, 22]. The majority of falsely positive results with regard to chronic alcohol abuse are seen in patients with the rare carbohydrate-deficient glycoprotein syndrome [7, 23–25] or with primary biliary cirrhosis [7, 20, 26, 27] and in healthy persons with genetic Tf-D variants [6, 7]. Excluding these subjects, CDT measurements are now used for clinical decisions in forensic and employment medicine and alcohol-related problems in traffic, and for screening for alcohol abuse as an exclusion criterion in patients with liver cirrhosis and undergoing liver transplantation [14, 28–30].

Within the scope of clinical evaluation of cCDT at our institute, we repeatedly found abnormal cCDT values despite a lack of alcohol overconsumption in patients who had undergone combined pancreas and kidney transplantation. Our aim in the present study was to investigate this clinical condition, kidney transplantation alone, and the corresponding immunosuppression as potential causes of abnormal cCDT values. Furthermore, we sought new insights into the pathomechanism of CDT increase, in addition to the increased sialidase activity and diminished activities of glycosyltransferases found by Xin et al. [31] in alcohol-treated rats and alcohol-abusing patients.

Here, we report for the first time above-normal cCDT and CDT/Tf ratios seen in male and female patients with combined pancreas and kidney transplantation despite no abnormal alcohol consumption. In contrast, patients with kidney transplantation alone or diabetics with end-stage nephropathy did not show increased cCDT or CDT/Tf ratios.

### Materials and Methods

MATERIALS

Pharmalytes 5–6 were obtained from Pharmacia/LKB (Freiburg, Germany); polyclonal antibodies (purified immunoglobulins) to Tf were from Dako (Hamburg, Germany); pure isotransferrin preparations were a gift of AXIS (Oslo, Norway). The remaining chemicals (all analytical grade) were obtained from Merck (Darmstadt, Germany).

SUBJECTS

All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. The reference subjects were 36 nonpregnant women (29 ± 10 years; mean ± SD), and 30 men (28 ± 9 years), all healthy subjects with an alcohol (ethanol) consumption of <20 g/day, who were recruited from the staff of our laboratory and from the medical student population at the University of Marburg (Marburg, Germany). Persons taking medication (other than oral contraceptives) were excluded from the study. Frequency, approximate daily total, and time of last alcohol intake were assessed by subjects' responses to a structured questionnaire [8, 32].

Six outpatients (three men and three women) with combined pancreas and kidney transplantation were studied, all with type I diabetes with end-stage nephrop-

athy. The kidney and pancreas were transplanted simultaneously from the same donor. A whole vascularized pancreas along with a segment of duodenum was implanted intraperitoneally, arterial blood being supplied from one iliac artery and venous drainage being directed into the ipsilateral iliac vein [33, 34]. The exocrine secretion from the graft was drained into the bladder (by means of pancreatico-duodeno-cystostomy). All patients had functioning pancreas and kidney grafts at the time of investigation, as checked regularly by routine clinical and laboratory analysis. None of them received insulin after transplantation. There were no clinical or laboratory signs of inflammation or sepsis, as indicated, e.g., by normal concentrations of C-reactive protein and neopterin in serum and physiological neopterin excretion in urine.

To assess possible effects of immunosuppression on cCDT, we also studied 16 patients with only kidney transplantation and functioning kidney grafts.

All transplanted patients had received a cadaveric allograft (pancreas and kidney or kidney alone) at the University of Marburg between 1991 and 1994. Characteristics of both groups of transplant patients, including immunosuppressive protocol and laboratory data, are given in Tables 1 and 2.

SERUM SAMPLES

To avoid additional blood drawing for the transplant patients, we used only surplus serum volumes obtained for routine investigations. For all subjects, blood was drawn after an overnight fast into sterile gel-tubes (Sarstedt, Nümbrecht, Germany). After the samples clotted at room temperature for 30 min, serum was obtained by centrifugation (2000*g*, 10 min, 4 °C). To avoid contaminating a sample with microorganisms, we removed the surplus sample volume with disposable pipettes and transferred it into a sterile, leakproof, 1.2-mL plastic container (Nalgene Cryotubes System 100; Nalge Co., Rochester, NY). Samples were immediately stored at −70 °C and were thawed only once for assaying. By this regimen, cCDT in the serum was stable for at least 6 months, as proved by additional use of these samples for analytical evaluation of the CDTect assay.

METHODS

*Assay of serum CDT.* Serum CDT was determined by CDTect-RIA (Pharmacia, Freiburg, Germany). After in vitro iron saturation of serum Tf and adsorption of isotransferrins with pI <5.7 on anion-exchange microcolumns, isotransferrins with pI >5.7 (CDT) were determined in the efflux by a competitive double-antibody RIA. CDT in the efflux competes with a fixed amount of ^{125}I-labeled Tf for the binding sites of the anti-Tf antibodies. Bound and free Tf were separated by addition of a second antibody as immunoadsorbent and centrifugation. The radioactivity in the pellet is inversely proportional to cCDT in the sample. Precision and accuracy of the assay were assessed by analysis of a serum pool and a quanti-

**Table 1. Clinical and laboratory data of patients with combined pancreas and kidney transplantation.**

| Patient, sex | Age at transplant, years | Time since transplant, months | Daily dose, mg | | | Serum and blood cell markers | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cyclosporine | Prednisolone | Azathioprine | CDT, U/L | Tf, g/L | CDT/Tf, % | γ-GT, U/L | MCV,[a] fL | Creatinine, mg/L | Glucose, g/L |
| Reference range | | | | | | | | | | | | |
|   Men | | | | | | 18[b] | 2.3–4.4 | 0.6[b] | 6–28 | 80–96 | 7–12 | 0.70–1.10 |
|   Women | | | | | | 28[b] | 2.3–4.4 | 1.0[b] | 4–18 | 80–96 | 6–11 | 0.70–1.10 |
| 1, man | 27 | 54 | 80 | 10.0 | 25 | 25 | 3.5 | 0.7 | 58 | 99 | 17 | 0.98 |
| | | 56 | 80 | 10.0 | 25 | 22 | 3.1 | 0.7 | 53 | 103 | 16 | 1.00 |
| | | 57 | 80 | 7.5 | 25 | 11 | n.d. | n.d. | 52 | 101 | 19 | 0.92 |
| | | 63 | 80 | 5.0 | 25 | 32 | 2.9 | 1.1 | 58 | 93 | 23 | n.d. |
| 2, woman | 38 | 54 | 150 | 7.5 | 0 | 31 | 3.0 | 1.0 | 212 | 87 | 47 | 0.87 |
| | | 55 | 150 | 7.5 | 0 | 31 | 3.7 | 0.8 | 145 | 87 | 48 | 1.47 |
| | | 57 | 150 | 7.5 | 0 | 10 | n.d. | n.d. | 247 | 89 | 41 | 0.92 |
| | | 58 | 125 | 7.5 | 0 | 12 | n.d. | n.d. | 180 | 87 | 47 | 1.52 |
| 3, woman | 33 | 48 | 320 | 7.5 | 75 | 37 | 2.7 | 1.4 | 7 | 99 | 20 | 0.81 |
| | | 50 | 320 | 7.5 | 75 | 45 | 2.8 | 1.6 | 5 | 95 | 15 | 1.00 |
| | | 52 | 320 | 7.5 | 50 | 15 | n.d. | n.d. | 6 | 95 | 16 | 1.01 |
| | | 55 | 320 | 7.5 | 50 | 33 | n.d. | n.d. | 5 | 96 | 14 | 0.93 |
| 4, woman | 31 | 23 | 225 | 7.5 | 75 | 45 | 3.7 | 1.2 | 20 | 98 | 19 | 1.08 |
| 5, man | 45 | 8 | 325 | 12.5 | 50 | 16 | 1.8 | 0.9 | 16 | 89 | 14 | 1.02 |
| | | 9 | 325 | 12.5 | 50 | 23 | 2.9 | 0.8 | 32 | 87 | 14 | 0.94 |
| | | 11 | 325 | 12.5 | 50 | 15 | n.d. | n.d. | 45 | 92 | 16 | 0.92 |
| | | 12 | 325 | 12.5 | 50 | 20 | 2.7 | 0.7 | 28 | 91 | 16 | 0.89 |
| | | 12 | 325 | 12.5 | 50 | 16 | n.d. | n.d. | 26 | 90 | 17 | 0.91 |
| | | 16 | 300 | 12.5 | 50 | 20 | 1.8 | 1.1 | 28 | 95 | 19 | 0.92 |
| 6, man | 37 | 7 | 250 | 15.0 | 50 | 24 | 3.9 | 0.6 | 17 | 71 | 13 | 1.22 |
| | | 7 | 200 | 15.0 | 50 | 42 | 4.4 | 1.0 | 18 | 72 | 15 | 1.04 |
| | | 9 | 200 | 12.5 | 50 | 11 | n.d. | n.d. | 14 | 73 | 16 | 1.25 |
| | | 11 | 200 | 12.5 | 50 | 14 | n.d. | n.d. | 14 | 78 | 16 | 1.27 |
| | | 16 | 200 | 10.0 | 50 | 31 | 2.9 | 1.1 | 13 | 91 | 15 | n.d. |

[a] Blood cell marker.
[b] 95th percentile upper reference limit.
n.d., not determined.

tative control sample (delivered with the test kit) with cCDT values near the upper reference limits for men and women in each run, according to the recommendations in the Guidelines of the Federal German Medical Association. Intra- and interassay variations were 10% and 17%, respectively. No external quantitative control sample was available. All measurements were done in duplicate and the mean was calculated.

The 95th percentile for cCDT and CDT/Tf ratio in 36 female and 30 male nondrinking or moderately drinking subjects was taken as the decision limit: 28 U/L and 1.0% in women, and 18 U/L and 0.6% in men. (The corresponding CDT values cited by the manufacturer are 26 and 20 U/L, respectively).

*IEF.* For qualitative confirmation of the CDTect results, we performed IEF according to Hackler et al. [4], using the PhastSystem[TM] (Pharmacia/LKB). The pH 5–6 polyacrylamide gels [total acrylamide content (T) = 5%, crosslinker content (C) = 3%; Pharmalytes 5–6® diluted 16-fold], adhering to a 43 × 50 × 0.45 mm plastic support film (Gel Bond[TM] PAG film; Biozym-Diagnostik, Hameln,

Germany), were prepared in the laboratory with a capillary casting mold similar to that described by Esen [35]. To reduce the number of Tf bands within the gel, we incubated the serum samples with ferric citrate solution, which yielded only $Fe_2$- but no $Fe_1$- and $Fe_0$-Tfs [4]. Also, we diluted the serum samples to equal Tf concentrations, to allow comparison of the intensity of the Tf bands in different lanes. Gels were placed on the cooling plate of the PhastSystem and prefocused for 75 Vh. We pipetted 1 $\mu$L of iron-saturated sample per lane (8 lanes available) onto the Sample Applicator[TM] 8/1 (Pharmacia/LKB), which was inserted into the sample applicator arm immediately after the "extra alarm." After the automated sample application, IEF was continued for a total of 200 Vh.

*Immunofixation.* Immunofixation was carried out as described by Hackler et al. [36, 37] with minor modifications. After removing the IEF gels from the cooling plate, we covered each gel with 150 $\mu$L of anti-Tf polyclonal antibodies [diluted threefold with 150 mmol/L NaCl solution (isotonic saline)]. The specificity of the Tf anti-

**Table 2. Characteristics of patients with kidney transplant only.**

| | Men | | Women | |
|---|---|---|---|---|
| | | Median (range) | | |
| No. of patients | 12 | | 4 | |
| Age at transplantation, years | 55 | (15–66) | 42 | (35–56) |
| Time since transplantation, months | 5 | (1–62) | 13 | (0.5–23) |
| Daily dose, mg | | | | |
|   Cyclosporine | | (225–400) | | (100–375) |
|   Prednisolone | | (5–20) | | (5–20) |
|   Azathioprine | | (25–50)[a] | | (25–75)[a] |
| Serum and blood measurements[b] | | | | |
|   CDT, U/L | 11 | (4–17) | 10 | (9–16) |
|   Tf, g/L | 2.5 | (1.8–3.8)[c] | 2.6 | (2.1–3.1)[a] |
|   CDT/Tf, % | 0.4 | (0.2–0.7)[c] | 0.4 | (0.3–0.5)[a] |
|   $\gamma$-GT, U/L | 10 | (7–134)[c] | 21 | (5–398)[a] |
|   MCV, fL | 89 | (76–95)[c] | 87 | (83–94)[a] |
|   Creatinine, mg/L | 15 | (12–45)[d] | 14 | (19–51) |
|   Glucose, g/L | 1.40 | (1.04–1.81)[c] | 0.89 | (0.44–1.74)[a] |

[a] n = 3 patients.
[b] For reference ranges see Table 1.
[c] n = 8 patients.
[d] n = 10 patients.

bodies was tested as described by Hackler et al. *[4]*. To achieve even distribution of the antibody solution, we covered the gel with a plastic foil the same size as the gel; after antibody incubation for 40 min at room temperature in a moist chamber, the foil was removed. Unprecipitated proteins were removed by washing the gel in isotonic saline with vigorous agitation at room temperature (20 °C). In all, 15–20 h of washing was required to thoroughly remove unprecipitated proteins; this was conveniently done overnight.

*Band visualization.* Silver staining of the isotransferrin bands was carried out in the PhastSystem Development Unit™ according to Hackler et al. *[37]*. The Tf bands were identified by comparison in each gel with identically treated isotransferrin standard preparations *[4]*, or by parallel analysis of a cerebrospinal fluid sample, showing asialo- to hexasialo-$Fe_2$-Tf bands (lane CSF in Fig. 1). Gels were air-dried and stored for documentation. In adjusting the sensitivity of the IEF for detection of CDTs (physiological concentration range usually 10–30 mg/L), we accepted an overload of the tetrasialo-$Fe_2$-Tf band as the main fraction (usually >2000 mg/L) (Fig. 1). Because the intensity of the tetrasialo-$Fe_2$-Tf band thus does not correlate with the amount of this isotransferrin present, measurement of the CDT/Tf ratio in the IEF gel is not possible.

*Other assays.* Serum $\gamma$-GT activity was measured at 25 °C with $\gamma$-glutamyl-4-nitroanilide as a substrate; serum creatinine was determined kinetically, without prior precipitation of proteins, by use of the Jaffe method; and serum glucose was analyzed by the hexokinase method, all with a Hitachi 747 analyzer and reagents from Boehringer Mannheim (Mannheim, Germany). Serum Tf concentration was determined immunonephelometrically with a Behring Nephelometer Analyzer (BNA; Behring, Marburg, Germany) and with reagents from the manufacturer. MCV was assessed with a MAXM-Hematology Analyzer (Coulter, Krefeld, Germany).

## Results
Clinical and laboratory data for patients with combined pancreas and kidney transplantation are summarized in Table 1. Despite no excessive alcohol consumption, cCDT was above the pertinent upper reference limit in patient 4 once and in the other patients repeatedly. Concentrations as great as 45 U/L were found in patients 3 and 4 (both women). A more than double increase of cCDT (42 U/L) was measured in patient 6 (a man). Abnormal CDT/Tf ratios between 0.7% and 1.1% for men and between 1.2 and 1.6% for women were found in 12 of 15 samples with increased cCDT. Owing to a reduced Tf concentration, an abnormal CDT/Tf ratio despite normal cCDT was found



Fig. 1. Serum $Fe_2$-isotransferrin IEF band pattern of patients with combined pancreas and kidney transplantation (*lanes A–E*: *A*, second investigation of patient 2 from Table 1; *B–D*, first investigations of patients 1, 3, and 5, respectively; *E*, second investigation of patient 6), and a healthy control proband (*F*).

Despite normal alcohol consumption in the transplant patients, the band patterns in *lanes A–C* and *E* are similar to those of patients with chronic alcohol abuse, showing intense disialo-$Fe_2$-Tf bands and additional mono- and asialo-$Fe_2$-Tf bands, corresponding to increased cCDT values obtained by the CDTect assay. The double bands in *lane D* are due to genetic transferrin polymorphism (C1–C2 phenotype), but sialic acid-deficient Tf bands are missing, as expected, from normal cCDT. *Lane CSF* shows the $Fe_2$-Tf band pattern of a human cerebrospinal fluid sample, which was used as an internal standard for identifying the various Tf bands. *Lane F* was used for comparing the intensity of the different isotransferrin bands between normal and pathological samples.
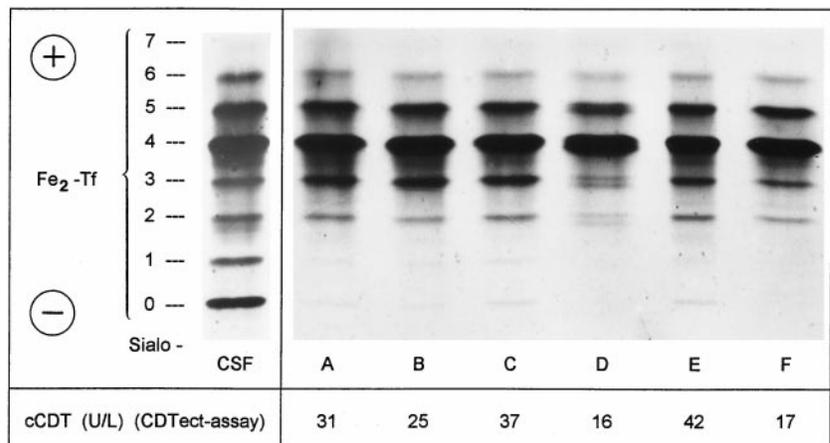
in one sample from patient 5. Increased serum Tf concentrations as a possible cause of the increase in cCDT were not seen in these patients (Table 1).

Except for patient 6, all patients with combined pancreas and kidney transplantation showed at least once an abnormal result for $\gamma$-GT or MCV, analytes often used as additional indicators of alcohol abuse. Both markers were above normal, however, only in patients 1 and 4, the latter showing only a slightly increased $\gamma$-GT activity. Increased $\gamma$-GT activities but normal MCV values were found in patients 2 and 5, the latter showing fluctuations of $\gamma$-GT between normal and abnormal values that did not correlate with cCDT. Increased MCV but normal $\gamma$-GT was measured in patient 3. We found no correlation between cCDT, CDT/Tf ratio, and $\gamma$-GT or MCV.

In contrast, none of the patients with kidney transplantation alone had an abnormal cCDT or CDT/Tf ratio or abnormal MCV (Table 2). Above-normal $\gamma$-GT activities were measured in only 3 of 16 patients.

In 20 poorly controlled diabetic patients (HbA$_{1c}$ >8%) with end-stage nephropathy who were admitted to the outpatient clinics for hemodialysis, no cCDT or CDT/Tf values were abnormal (data not shown).

To exclude as causes of abnormal cCDT specific analytical errors, e.g., abnormal chromatographic behavior of the isotransferrins of patients with combined pancreas and kidney transplantation or interassay variations of the CDTect assay, we analyzed all serum samples for CDT by a second, independent method: qualitative IEF (Fig. 1). Lane CSF of Fig. 1 shows a typical isotransferrin pattern of cerebrospinal fluid, which we used to identify the isotransferrin bands in lanes A-F. From anode to cathode, the fractions are a-, mono-, di-, tri-, tetra- (main fraction), penta-, and hexasialo-Fe$_2$-Tf. The isotransferrin pattern in lane F (from anode to cathode: di-, tri-, tetra-, penta-, and hexasialo-Fe$_2$-Tf) is typical of healthy Caucasians with normal alcohol consumption. We ran with each gel an aliquot of this typical serum, which allowed us to identify altered isotransferrin patterns. Lanes A-E of Fig. 1 represent isotransferrin band patterns found in patients with combined pancreas and kidney transplantation. The isotransferrin patterns within lanes A-C and E are similar to those of patients with chronic alcohol abuse, i.e., intense disialo- and additional mono- and asialo-Fe$_2$-Tf bands [4]. The corresponding cCDT values obtained by CDTect-RIA were also increased, whereas serum of lane D had a normal cCDT value and a normal isotransferrin band pattern. The double bands in lane D reflect the genetic Tf-C polymorphism (C$_1$–C$_2$ phenotype).

Other patients—diabetics or patients with kidney transplantation alone—did not show isotransferrin patterns similar to those in lanes A-C and E. This confirms the normal cCDT values found in these patients (Table 2).

## Discussion

In this study, increased cCDT and CDT/Tf ratios surpassing the gender-specific upper reference limits for diagno-

sis of chronic alcohol abuse were found in more than half of the serum samples of all patients with combined pancreas and kidney transplantation.

The reason for increased cCDT and CDT/Tf ratios as well as for individual fluctuations of these markers remains unclear, given the absence of any change in transplant function, clinical background, medication, or alcohol intake. Although all samples from controls, diabetics, and transplant patients were collected and treated in the same way, increased cCDT was observed only in patients with combined pancreas and kidney transplantation. Some of these samples were reanalyzed after 6 months and some after repeated thawing, but the cCDT results remained the same. Contamination of the serum samples, e.g., with sialidase-producing microorganisms, is therefore unlikely as a cause for increased cCDT. A microbial infection of the transplant patients is also improbable: Regular measurements of serum C-reactive protein as an immediate and sensitive indicator of inflammation [38] and regular assays of neopterin in serum and urine as very early and sensitive markers for most forms of infection in transplant patients [39] yielded normal results. Analytical errors could be excluded as causes for increased cCDT in each case because the corresponding IEF isotransferrin band pattern showed distinct bands of sialic acid-deficient transferrins similar to those seen in alcoholics (Fig. 1). The interassay CV of 17% for cCDT could not account for the cCDT fluctuations shown here between consecutive measurements, which were always done in duplicate. Sorvajarvi et al. [40] recently reported a marked decrease of the diagnostic specificity of cCDT in patients with increased serum concentrations of Tf; however, this specificity could be improved by use of %CDT values. For the patients with combined pancreas and kidney transplantation, no increased serum Tf concentration was measured, thus excluding this condition as a cause of increased cCDT. Furthermore, when we calculated the CDT/Tf ratios of 15 samples with increased cCDT, 12 samples showed above-normal values, 1 had normal values, and 2 had the ratio at the upper reference limit. Moreover, one patient had an increased CDT/Tf ratio despite having a normal value for cCDT. Thus, using the CDT/Tf ratio did not improve the diagnostic specificity in the transplant patients.

In view of history, close surveillance, and self-reports stating a daily ethanol consumption of <20 g, chronic alcohol abuse is unlikely as a cause of increased cCDT in each of the transplant patients. However, $\gamma$-GT activity and MCV as potential indicators of alcohol abuse were increased in several of these patients' samples (Table 1). Hepatotoxic effects of cyclosporine and of azathioprine are well documented [41]. The latter, metabolized to 6-mercaptopurine (which acts as a purine antagonist), inhibits DNA synthesis and thus the proliferation of fast-growing cells, such as granulocytes, erythrocytes, and platelets [41, 42]. Given this mechanism, and with normal erythropoietin synthesis in kidney transplant recipients,

macrocytemia may occur as a side effect of azathioprine therapy, a fact that is often used for monitoring patients' compliance [42]. A deficiency in thiopurine *S*-methyltransferase (EC 2.1.1.67; an enzyme involved in 6-mercaptopurine metabolization that exhibits a wide range of activity in the normal population [43]) must also be taken into account as a cause of increased MCV. It is, however, unlikely that all patients with combined pancreas and kidney transplantation would have this disorder. Summing up, abnormal MCV and increased $\gamma$-GT activities most probably result from the medication rather than increased alcohol intake.

Initially, we assumed that the increase in cCDT was a side effect of the immunosuppressive medication, so we measured cCDT in 16 patients after kidney transplantation alone and treatment with similar basic immunosuppressive regimens (Table 2). None showed an increased cCDT or CDT/Tf ratio (Table 2). Medication could therefore hardly cause increased cCDT in patients with combined pancreas and kidney transplantation. Interestingly, above-normal MCV was not observed in patients with kidney transplantation alone, even though six of them received azathioprine. This could result from: (*a*) a statistical effect related to the small number of patients, (*b*) the different hormonal situation in the two groups of patients (see below), and (or) (*c*) the different metabolic situation, e.g., hyperglycemia in patients with kidney transplantation alone vs normoglycemia in patients with combined pancreas and kidney transplantation. To our knowledge, whether these factors exacerbate or mitigate the azathioprine side effects is unknown.

A causal role by the underlying disease of type I diabetes mellitus for increased cCDT is also unlikely. On the one hand, the metabolic situation was effectively treated in all of the patients with combined pancreas and kidney transplantation, as indicated by, e.g., generally normal serum glucose concentrations (Table 1). On the other hand, 20 sera from poorly controlled diabetic outpatients on hemodialysis ($HbA_{1c} > 8\%$) did not show increased cCDT or abnormal CDT/Tf ratios. To our knowledge, increased cCDT in diabetics has not been reported. A correlation between cCDT and renal function, i.e., as expressed by the serum creatinine concentration (Tables 1 and 2), was not observed in either transplant patient group.

The implantation of the pancreas allograft remains the main difference between the patient groups. According to present knowledge, the pancreas itself does not play any important role in the metabolism and turnover of transferrin and CDT. Successful pancreas transplantation results in a carbohydrate metabolism similar to that in nondiabetic subjects [44, 45]. However, the drainage of the pancreatic venous effluent into the systemic venous system instead of the portal venous system inevitably induces systemic hyperinsulinemia [46, 47], owing to reduced insulin clearance by the liver [46]. This hyperinsulinemia is exacerbated by the steroid (e.g., prednisolone)-induced peripheral insulin resistance [48], and we can speculate that these conditions may alter Tf and CDT turnover. The peripheral insulin resistance should develop within the duration of systemic hyperinsulinemia [46, 48]. This may explain why 20 serum samples drawn from patient 4 within the first 60 days after simultaneous pancreas and kidney transplantation showed normal cCDT values (preliminary results), whereas ~2 years after transplantation her cCDT had increased to 45 U/L (Table 1). In this context, the findings of Fagerberg et al. [49] concerning a relation between insulin sensitivity and cCDT need further investigation. Besides hyperinsulinemia and normoglycemia, patients with combined pancreas and kidney transplantation differ from patients with kidney transplantation alone by having improved lipid metabolism [50]. Which factor or hormone–substrate interaction is responsible for increased cCDT in patients with combined pancreas and kidney transplantation remains unclear.

In conclusion, combined pancreas and kidney transplantation must be considered as a new cause of above-normal cCDT and CDT/Tf ratios despite normal alcohol consumption. Owing to the increasing number of studies reporting pathological cCDT values despite normal alcohol intake in various diseases [7, 13, 19, 20, 22], a synopsis of clinical data and of suitable laboratory markers is a prerequisite for diagnosis of alcohol abuse.

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