tifies 85% of the alleles, is rapid and inexpensive, and may be used in all laboratories without specific qualification requirements. Our diagnostic strategy for hereditary fructose intolerance is specific to our population but can be transposed and adapted to other populations in which different mutant alleles of aldolase B contribute to the burden of disease. In the future, the development of automated mutation detection with oligonucleotide arrays on chips available for rare disorders (13) will simplify the molecular diagnosis of HFI.

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References

Increased Serum Lipase with Associated Normoamylasemia in Cancer Patients, Giampiero Diani,1* Giuseppe Poma,2 Francesco Novazzi,1 Sonia Zanirato,2 Camillo Porta,2 Mauro Moroni,3 Gian Vico Melzi d’Eril3 and Remigio Moratti1 (1 Laboratorio di Analisi Chimico-cliniche and 2 Dipartimento di Medicina Interna e Terapia Medica, Sezione di Medicina Interna e Nefrologia, Università degli Studi di Pavia, Instituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo, I-27100 Pavia, Italy, and 3 Laboratorio di Analisi Chimico-cliniche, Ospedale di Circolo, Varese, Italy; * address for correspondence: Laboratorio di Analisi Chimico-cliniche, Instituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo, I-27100 Pavia, Italy)

Measurements of the activity of both lipase (triacylglycerolacylhydrolase, EC 3.1.1.3) and α-amylase (EC 3.2.1.1) in serum are commonly used as aids in the diagnosis and follow-up of acute and recurrent pancreatitis (1); 19% of pancreaticit patients present with normal serum amylase (2), thus leaving lipase as the main hematocchemical marker of this disease in a substantial number of patients.

Lipase and amylase are increased also in conditions other than pancreatitis, such as several nonmalignant hepatobiliary and gastrointestinal diseases, sepsis, renal failure, pulmonary failure, and subdural bleeding (1–4).

In 1987, Stein et al. (5) reported on a non-Hodgkin’s lymphoma patient with major lipase activity (up to seven times the upper reference value), with no parallel increase in amylase; in this case, the authors demonstrated a specific immunoglobulin G with high affinity for lipase as the cause of increased serum lipase activity.

More recently, Donnelly et al. (6) have reported a chronic increase in serum lipase, again with no associated hyperamylasemia or clinical evidence of pancreatitis, which the authors suggested to be tumor-derived; furthermore, Muñoz-Perez et al. (7), after observing chronic increased serum lipase in another patient with massive abdominal metastases from a suspected pancreatic adenocarcinoma, suggested that the biochemical profile characterized by increased lipase and normal amylase could be an early finding of a malignant neoplasm.

We report on four cases in which serum lipase increase first anticipated an otherwise undetectable tumor relapse or progression.

Serum lipase activity was measured with a commercially available kinetic colorimetric co-lipase method (Lipase KC, Bayer Italia S.p.A., Divisione Diagnostici). Briefly, in the assay, pancreatic lipase catalyzes the hydrolysis of the 1,2-diglyceride sulfate to produce fatty acids and 2-monoglyceride. The latter, in the presence of specific auxiliary enzyme, release glycerol, which is then converted, by glycerokinase, into glycerol-3-phosphate and hydrogen peroxide. Finally, in the presence of peroxidase, the H2O2 delivered oxidizes the chromogen system to a violet dye. The mean value of the absorbance (A550) changes per minute is calculated. The reference interval for serum was 8–57 U/L at 37 °C (8).

CASE #1
A 61-year-old man was diagnosed in December 1992 with malignant rectosigmoid adenocarcinoma after mild anemia was observed. Early infiltration of the vesical dome was found at subsequent anterior resection; the whole mass was ablated, and the patient was started on postoperative irradiation and adjuvant chemotherapy. After treatment, the patient remained free of evidence of disease till May 1994, when abdominal computed tomography showed liver metastases. His CA 19.9 was 109 IU/L (normal reference values, ≤37 IU/L). Advanced systemic chemotherapy, started in June 1994, controlled the growth of metastases. In November 1995, abdominal ultrasonography (US) showed stable liver findings and no pancreatic or extrahepatic biliary involvement. Hematocchemical tests confirmed the previous findings of hepatocellular
injury (150 U/L aspartate aminotransferase and 164 U/L alanine aminotransferase), high lipase (170 U/L; normal reference values, 8–57 U/L), normal amylase, and CA 19.9 of 37 IU/L.

The treatment was then discontinued on the patient’s request, mainly because the disease was stable. In January 1996, abdominal US showed marked disease progression in the liver, with many new secondary lesions. Lipase remained high (197 U/L), amylase was normal, and CA 19.9 had increased again (215 IU/L). The patient then received second-line chemotherapy, and he is still alive.

**CASE #2**

A barium enema on a 52-year-old woman in April 1994, identified lesions in the colon at the hepatic flexure. Biopsy during colonscopy permitted the histologic diagnosis of colon adenocarcinoma. After resection and with distant metastases excluded, the patient was staged as C2 according to Dukes’ classification, modified by Astler and Coller (9); she then received six cycles of adjuvant combination chemotherapy. Before surgery, CA 19.9 was increased (324.67 IU/L).

After adjuvant treatment, instrumental and hematological findings, including CA 19.9 titration, were normal. However, high lipase (120 U/L) was first found in May 1995, whereas amylase and tumor markers remained normal.

Three months later, CA 19.9 was markedly increased (94 IU/L). Subsequent liver US showed a liver metastasis. Lipase titers remained high, whereas amylase was unchanged. Laparotomy was then performed to resect the metastasis and to position a small intrahepatic catheter; incidentally, no objective signs of pancreatitis were found during the surgical procedure. Subsequently, the patient started on locoregional chemotherapy but died 9 months later of cancer-related liver failure.

**CASE #3**

Hepatocellular carcinoma was suspected at liver US performed in January 1995, in a 64-year-old woman with previous hepatitis B and C, and carcinoma was confirmed at subsequent US-guided needle biopsy. The patient was inoperable because of multifocal cancer and poor liver function (29 g/L albumin and 870 mg/L cholesterol; prothrombin time was 42% of control), and she therefore was started on a course of combination chemotherapy. At that time, both amylase and lipase were within the reference intervals (215 and 44 U/L, respectively), whereas CA 19.9 and α₁-fetoprotein (α₁-FP) were high (150 and 92 U/L, respectively). Instrumental tests after chemotherapy cycle 3 showed complete response, with no more signs of disease appearing at computed tomography. CA 19.9 was markedly lower (37 IU/L), and α₁-FP concentration was normal (7 U/L). Subsequent US performed 4 weeks after computed tomography confirmed disease remission. Three months later, US showed no sign of disease progression, but lipase was high (242 U/L), even with no other biohumoral changes; particularly, CA 19.9 and α₁-FP titers were much the same as in the former tests (44 U/L and 11 U/L, respectively), whereas amylase remained in the normal range (220 U/L). US performed 1.5 months later because of severe jaundice and abdominal pain showed marked disease progression in the liver. Lipase was again high (351 U/L), amylase was unchanged, and tumor markers were positive again (CA 19.9 was 157 U/L and α₁-FP was 397 U/L).

The patient died of liver failure three weeks later. No signs of pancreatitis were evidenced at autopsy.

**CASE #4**

A 64-year-old chronic alcoholic and hepatitis C virus-positive hepatopathic man was referred to the Otorhino-laryngology Department of our Hospital in February, 1995, because of mainly inspiratory laryngeal dyspnea. A subglottic laryngeal cancer (T₂) with bilateral lymph nodes (N₂) but no distant (Mₒ) metastases was diagnosed. Preoperative tests showed high lipase (318 U/L) and no hyperamylasemia (180 U/L amyase). Other hematological tests showed only increased γGT (87 U/L) and alkaline phosphatase (344 U/L). Subsequent abdominal US showed no neoplastic evolution and no gross focal lesions in the pancreatico-bilio-choledochal region, which was, however, partly masked by abdominal gas. In March 1995, the patient underwent total laryngectomy with bilateral functional emptying and then was started on adjuvant irradiation, including the anterior mediastinum. Major signs of hepatocellular necrosis were found at 3 months of follow-up (136 U/L aspartate aminotransferase and 124 U/L alanine aminotransferase), together with worsening γGT (112 U/L) and alkaline phosphatase (416 U/L). Once again, no hyperamylasemia was found, but lipase was high (412 U/L); tumor marker assessment showed high CA 19.9 only (69 IU/L). Subsequent abdominal US depicted a focal liver lesion, ~2 cm in diameter, which needle biopsy proved to be a well-differentiated hepatocellular carcinoma with cholangiocellular differentiation areas. The patient then underwent US-guided percutaneous alcoholization; no signs of disease progression in the liver or recurrence in the head and neck have been observed since then.

Serum lipase activity increased in all our patients without a parallel increase in serum amylase titers; the combination of accurate clinical, biochemical, and instrumental study of these patients allowed us to exclude the possibility of a normoamylasemic pancreatic injury, and the presence of appropriate concentrations of bile salts and colipase in the reagent favors a full and specific action of lipase assay, at the same time inhibiting serum esterase (8). Furthermore, not one of the above patients had fat emboli or received large heparin doses, conditions that could have interfered with lipase titration.

An increase in lipase activity preceded the progression or relapse of a previously known colorectal or hepatic cancer in three cases; in the extant patient, increased lipase titers, observed during the staging of a laryngeal cancer and not decreasing after the surgical removal of the primary head and neck tumor, preceded the development
of a subsequent second neoplasm, a primary hepatocellular carcinoma.

Furthermore, an increase in lipase activity preceded, by an average of 2.6 months (4, 1.5, 3, and 2 months, respectively), the increase of specific tumor markers, such as CA 19.9 and α1-FP.

Several possible mechanisms may explain this unexpected increase in lipase activity, such as the presence of an unusual lipase isoenzyme, a decrease in the clearance of lipase or rate of its inactivation in the circulation, the persistence of lipase in blood after complexation of lipase with a plasma protein (5), or the direct production of lipase from the neoplastic mass, as suggested by Donnelly et al. (10) in a patient with a retroperitoneal malignancy, and by Lopez-Soriano et al. (10).

Several authors claim that the nonspecificity of lipase assays for pancreatitis is due to the pancreas being sensitive to other abdominal disease; as a matter of fact, the possibility that tumors may produce humoral factors influencing pancreatic metabolism and/or lipolytic activity is supported by a recent study suggesting that tumor necrosis factor-α is involved in activating the lipid metabolic changes that develop in rats after transplantation of a fast-growing tumor (11).

Although we did not investigate which of the above mechanisms was involved in our patients, we consider intriguing the hypothesis of a tumor marker-like role for lipase; indeed, in our experience, lipase acted as an extremely precocious signal of malignancy in all patients, anticipating the increase of more common tumor markers, such as CA 19.9 and α1-FP.

However, only prospective studies on an adequate number of patients could definitely assess the real role of lipase from this point of view.

References

An improved method for the detection of the thermolabile variant of methylene tetrahydrofolate reductase, Geneviève Van Amerongen,1 Florence Mathonnet,1,2 Catherine Boucly,1 Bertille Mathieu,1 Isabelle Vinatier,1 Jean-Yves Pel- lier,6 Nicole Catherine,1 Catherine Collet,1 and Philippe de Mazencourt4,5,6 (1 Laboratoire de biochimie et biologie moléculaire, Hôpital R. Poincaré, F92380 Garches, France; 2 Laboratoires d’hématologie du centre hospitalier de Poissy-Saint Germain en Laye, F78303 Poissy, France; 3 Service de Médecine Interne, centre hospitalier de Poissy-Saint Germain en Laye, F78303 Poissy, France; 4 Faculté de Médecine Paris Ouest, CJI 9402, Université Paris, V, F92380 Garches, France; * author for correspondence: fax 331 47 10 79 23)

The thermolabile variant of the methylene tetrahydrofolate reductase (MTHFR) in the homozygous state has been shown to be responsible for mild hyperhomocystinemia, hypomethioninemia, and hyperhomocystinuria (1). This variant is responsible for an increased risk for recurrent early pregnancy loss and neural-tube defects (2,3). The presence of hyperhomocystinemia is also predictive of both arterial and venous thromboembolic disease (4–7) and is a risk factor for coronary artery stenosis, independent of other risk factors such as age, smoking, hypercholesterolemia, and hypertension (8). Four to 6% of the Caucasian population (9) and 13–20% of the thrombosis-prone patients are homozygous for the thermolabile variant of MTHFR, which is caused by a C-to-T substitution at nucleotide 677 of the cDNA, resulting in the substitution of a valine for an alanine (8). A simple molecular diagnosis is of particular interest because this risk factor is quite common, the biochemical assay requires a methionine load, and folate supplementation is likely to prevent some of the complications (10,11). Thus, the exploration has been recommended in the management of premature venous and arterial occlusive diseases (4). We report here an improvement of the method described previously to assess the thermolabile variant of MTHFR, based on multiplex amplification of MTHFR and an internal control.

We studied 30 healthy control volunteers and 30 patients with personal or familial history of thrombosis or phlebitis. Informed written consent was obtained in all cases.

DNA extraction was performed from frozen blood either by phenol-chloroform extraction according to McIndoe et al. (12) or with DNAzol (Life Technologies, Inc.) as recommended by the manufacturer. In most of the control subjects, DNA was extracted with DNAzol from the cell pellet of saliva after two washes in 9 g/L NaCl.

Amplification of MTHFR was adapted from the method described by Froost et al. (1) as follows. Initial denaturation step was for 4 min at 94 °C followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 62 °C, and elongation for 90 s at 72 °C. The final elongation step was for 12 min at 72 °C. Primers used were: primer A, 5′-TGA AGG AGA TGT CTC CGG GA; primer B, 5′-AGG ACG GTG CGG TGA GAG TG; primer C, 5′-CTC