CME/SAM

Frequency of Pancreatic Hyperamylasemia in Human Immunodeficiency Virus–Positive Patients in the Highly Active Antiretroviral Therapy Era

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ABSTRACT

Objectives: *Increased frequency of hyperamylasemia has previously been reported in human immunodeficiency virus (HIV)-positive patients, but studies determined total amylase activity and were performed before the introduction of highly active antiretroviral therapy (HAART). We evaluated the frequency of pancreatic hyperamylasemia in a large HIV+ population mostly treated with HAART.*

Methods: *The upper reference limit (URL) for pancreatic amylase (P-AMY) was derived from 299 healthy blood donors. A cross-sectional study was then performed on samples obtained from 1,548 consecutive patients referred to our infectious disease clinic to assess serum P-AMY and lipase concentrations. Of the patients, 94% were HIV+, and most (92%) were taking HAART (HIV+Tx+).*

Results: *P-AMY URL was 51 U/L. The frequency of P-AMY increase did not significantly differ between HIV+ and HIV - populations (14.2% vs 15.2%, P = .91) or between HIV+Tx+ and HIV+Tx - (14.7% vs 8.9%, P = .11). In almost half (48.3% of HIV+ and 42.9% of HIV -) of hyperamylasemic patients, lipase was normal, indicating a non pancreatic origin of their P-AMY increase. Markedly elevated P-AMY (>3 times the URL) was found in six HIV+ patients and in one HIV - patient: two had macroamylasemia, one acute pancreatitis, three (including the HIV - patient) chronic pancreatitis, and one chronic hyperamylasemia of undefined origin.*

Conclusions: *In our study, both HIV+ and HIV+Tx+ do not show an increased frequency of P-AMY elevation. Frank pancreatic disease is rare in this clinical setting.*

Upon completion of this activity you will be able to:

- describe problems regarding the definition of biochemical pancreatic abnormalities used in studies aimed to estimate the frequency of pancreatic involvement in human immunodeficiency virus (HIV)–positive patients.
- predict the approximate frequency of pancreatic hyperamylasemia in HIV+ patients with and without highly active antiretroviral therapy at different enzyme cutoffs (ie, more than the upper reference limit [URL] and more than three times the URL).
- synthesize the reasons why the pancreatic amylase measurement cannot be recommended as a screening test for detecting pancreatic injury in asymptomatic HIV+ patients.

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Historically, several factors (eg, opportunistic infections, AIDS-related cancer and antineoplastic chemotherapy, and use of nucleoside analogues or antimicrobials such as pentamidine, cotrimoxazole, and antimycobacterial compounds) were suspected to cause acute or chronic pancreatic injury in patients with human immunodeficiency virus (HIV) infection (HIV+).[1] Even after the introduction of highly active antiretroviral therapy (HAART) in 1996, HIV+ patients continued to be exposed to multiple potential risk factors for pancreatic involvement, including the

administration of pancreotoxic drugs, alcohol or illicit substance abuse, antiretroviral therapy–related hypertriglyceridemia, concomitant chronic liver and/or biliary disorders, opportunistic diseases and HIV infection itself, and the progressive increase of patients' age and related comorbidities.[2] Nonetheless, the frequency of signs and symptoms of pancreatic injury has remained relatively low, and full-blown acute pancreatitis has continued to be a rare clinical condition in HIV+ patients through the years.[3,4] Reisler et al[5,6] reported an overall acute pancreatitis rate of 0.61 per 100 person-years among 8,451 participants enrolled in 20 AIDS clinical trials group studies from 1989 to 1999 and detected pancreatitis in 0.85 per 100 person-years between 1996 and 2001 in 2,947 HIV+ patients taking HAART, while the EuroSIDA study involving 9,678 HIV+ patients observed a lower overall rate (0.13 cases per 100 person-years) in the 2001 to 2006 period.[7] Although these data confirm that acute pancreatitis in the HIV+ population is not a frequent disease, its incidence seems notably higher than the annual rate of acute pancreatitis in ostensibly healthy people (0.02-0.03 cases per 100).[8]

It is important to note that, in estimating the frequency of acute pancreatitis, the employed diagnostic criteria are crucial; for instance, in the first of the above-mentioned studies,[4] the use of clinical and/or laboratory criteria yielded a pancreatitis rate nearly fourfold higher than that obtained using only clinical diagnosis. On the other hand, the incidence of isolated biochemical pancreatic abnormalities showed an extended range of frequency in the pre-HAART era, generally much higher than the frequency of laboratory and/or clinically diagnosed pancreatitis.[1,9] Even in more recent studies, neither diagnostic criteria for pancreatitis nor the definition of *biochemical pancreatic abnormalities* are unequivocal, making it difficult to compare frequency data. In one study that examined the frequency of acute pancreatitis in a larger group of HIV+ individuals treated in Northern California during the same period (2001-2006) than did the EuroSIDA study, but using different diagnostic criteria, the annual incidence of pancreatitis was approximately five times higher than that in the EuroSIDA cohort.[10] It is also apparent that different diagnostic criteria are not the only factors influencing pancreatitis frequency in published studies. Differences in diet and alcohol consumption, the frequency of hepatitis C virus (HCV) coinfection, and drugs used beside antiretroviral agents have been adduced.

Acute pancreatitis is sometimes difficult to diagnose, and measurements of pancreatic enzymes have a fundamental role.[11] The American Gastroenterological Association and the American College of Gastroenterology recommend that the diagnosis of acute pancreatitis should be based on compatible clinical features and, in the absence of renal failure, a more than three times the upper reference limit (URL) elevation of amylase (AMY) or lipase concentrations in serum.[12,13] Elevation of lipase levels is somewhat more specific and is thus preferred. Fewer than 3 times the URL elevations of pancreatic enzymes have low specificity for acute pancreatitis.[14,15]

Lack of specificity of total AMY determination has led to interest in the direct measurement of pancreatic AMY (P-AMY) isoenzyme instead of total enzyme activity in serum for the differential diagnosis of patients with acute abdominal pain.[11,14] By applying the best decision limit (ie, >3 times the URL), the clinical specificity of P-AMY for the diagnosis of acute pancreatitis is more than 90%.[11] The evaluation of clinical significance of elevated P-AMY concentrations in serum is sometimes complicated by the presence of macroamylase (MA), a complex of AMY and immunoglobulin G or immunoglobulin A, which is not of pathologic significance.[11] The presence of MA has been reported in HIV+ patients, and this may confound their clinical evaluation.[9,16,17] Another interesting question is whether there is any difference in the frequency of acute pancreatitis and of biochemical pancreatic abnormalities between the pre-HAART and the HAART era.

In this study, we aimed to evaluate the frequency and the degree of pancreatic hyperamylasemia in a large HIV+ population mostly treated with HAART. Increased frequency of hyperamylasemia has been reported in HIV+ patients, but most of the studies determined total AMY activity and applied nonstandard criteria for the definition of biochemical pancreatic abnormalities.[1,9,18] Furthermore, most studies were performed before the introduction of HAART, so possible changes in the frequency of hyperamylasemia and acute pancreatitis in HIV+ patients due to the introduction of HAART need to be evaluated.

Materials and Methods

Analytical Methods

Serum P-AMY and pancreatic lipase concentrations were measured using enzymatic colorimetric assays on the cobas 6000 platform (Roche Diagnostics, Basel, Switzerland).[19,20] Stability of both enzyme measurements was monitored during the entire study period by the internal quality control (IQC) performed daily using fresh-frozen serum material (Liquicheck Unassayed Chemistry Control Level 2, lot no. 16672; Bio-Rad, Hercules, CA): results showed an average coefficient of variation (CV) of 1.15% for P-AMY (cumulative mean, 324 U/L) and an average CV of 1.85% for lipase (cumulative mean, 57 U/L), respectively.

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According to Braga et al,[21] this type of IQC provides, through mechanisms of retrospective evaluation, data useful for the knowledge of variability of the analytical system (eg, the reagent lot-to-lot variation) and of its daily use by the individual laboratory. During the clinical study, in all patients with P-AMY more than the URL, the presence of MA was assessed using the polyethylene glycol (PEG) precipitation method (PEG-6000 concentration, 240 g/L).[22] The presence of MA was confirmed if the P-AMY residual activity in the supernatant was 35% or less.[23]

Reference Sample Groups

URLs of serum P-AMY, lipase, and P-AMY/lipase ratio (A/L) were experimentally determined in two separate studies. In a first study, aiming to establish the URL for P-AMY, serum samples were obtained from 300 apparently healthy blood donors (150 men and 150 women, aged 18-65 years). In a second study, performed on a different group of 120 healthy blood donors (60 men and 60 women, mean ± SD age of 43.7 ± 13.7 years), the URL for A/L was determined and the previously established URL for P-AMY (first study) and lipase[24] verified. A/L estimate was considered useful in showing the origin of the enzyme release, since a pancreatic injury should cause the contemporaneous release of both enzymes, with the ratio basically unaltered, whereas other nonpancreatic causes of pancreatic hyperamylasemia (eg, MA) not influencing lipase concentrations should markedly increase A/L. Reference samples were analyzed on the same day of blood collection.

Clinical Study

Afterward, a cross-sectional prospective study was performed (enrollment period, March-August 2013) by measuring P-AMY and lipase on blood samples obtained from 1,548 consecutive participants (71.7% male; median age, 48 years [range, 21-89 years]) referred to our infectious disease inpatient clinic for evaluation. Blood samples were drawn in the clinic and immediately sent to the laboratory, where they were processed and determined for pancreatic enzymes. An independent researcher (A.R.), blinded to laboratory results, retrieved data from clinical records related to all enrolled patients. The study was done in compliance with the Helsinki Declaration of 1975, as revised in 2008, and informed consent of participants was obtained.

Statistical Analysis

Reference intervals were derived according to the Clinical and Laboratory Standards Institute C28-A3 protocol.[25] Reed's criterion was used for outlier detection and the Shapiro-Wilk test for testing normality of data distribution. The 95% central reference interval was determined by the nonparametric statistical method. Hyperamylasemia and hyperlipasemia frequency data were compared between different patient groups using the χ^2 test. A value of $P < .05$ was considered statistically significant. All statistical evaluations were performed using the MedCalc (Ostend, Belgium) v.12.7.5 software package.

Results

In the first substudy, aimed to determine the reference interval for P-AMY, one outlier result from 300 samples was detected and excluded from the analysis. Using the nonparametric statistical method (Shapiro-Wilk test rejected normality of data distribution, $P < .0001$), the following reference interval (90% confidence interval in parentheses) was found for P-AMY in serum: 13 (11-13) U/L to 51 (46-54) U/L, with no sex difference. These results were confirmed (ie, 12-50 U/L) in the second substudy, analyzing the P-AMY concentrations of 120 additional blood donors. Furthermore, results were quite similar to those previously determined by Junge et al[26] using the same method on a different population (13-53 U/L, n = 775). Consequently, 51 U/L was henceforth applied in our study as the URL for P-AMY to define pancreatic hyperamylasemia in the following clinical study. From data of the second reference substudy, we first derived the lipase reference interval (data not normally distributed, $P < .0001$) as 17 U/L (12-19) to 64 U/L (53-104) and then calculated the A/L reference interval as 0.40 (0.36-0.48) to 1.73 (1.37-2.13).

In the clinical study, 1,456 (94%) of 1,548 patients were HIV+, with most (92%) receiving HAART therapy **Table 1**. The remaining 92 (6%) patients were HIV–, affected by conditions such as HCV or hepatitis B virus infection (n = 58), other liver disease (n = 5), rheumatic disease (n = 6), nonviral infections (n = 13), and other miscellaneous conditions (n = 10). Serum P-AMY more than the URL was found in 14.3% of enrolled patients, and the frequency of pancreatic hyperamylasemia did not significantly differ between HIV+ and HIV– populations (14.2% vs 15.2%, $P = .91$) or between HIV+ patients taking HAART and those who did not (14.7% vs 8.9%, $P = .11$). In almost half (48.3% of HIV+ and 42.9% of HIV– patients, $P = .91$) of hyperamylasemic patients, lipase concentrations were normal (≤64 U/L), indicating a probable nonpancreatic origin of their P-AMY increase. However, in only 8 of these patients (all HIV+) was MA accountable for hyperamylasemia **Table 2**. Although no patients with concomitant hyperamylasemia and hyperlipasemia (ie, P-AMY >51 U/L and lipase >64 U/L) had MA detected, in two MA cases, A/L remained less than 1.73 and failed to recognize the

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condition, indicating that this ratio has a limited role in screening MA.

Markedly elevated P-AMY concentrations (>3 times the URL) were found in only six HIV+ patients and one HIV– patient Figure 1 and Table 3: two had MA (displaying the highest P-AMY activities), one acute pancreatitis, three (including the HIV– patient) chronic pancreatitis, and one chronic pancreatic hyperenzymemia of undefined origin.

Discussion

Pancreatic injury found in HIV+ patients during the pre-HAART era generally resulted from an extended exposure to multiple medications and/or concurrent AIDS-related disorders. The spectrum of potential risk factors for pancreotoxicity has changed but not diminished with the introduction of HAART, which has significantly modified the natural history and outcomes of HIV disease, transforming it into a predominantly chronic, treatable disorder characterized by a sharp drop of immunodeficiency-related complications and a concurrent increase of drug-associated long-term toxicity.[2] Studies evaluating the presence of hyperamylasemia in HIV+ patients before the introduction of HAART showed an increased frequency of this condition,[1,9,16,18] even if its amount was dependent on the employed detection criteria. For instance, in the small study by Argiris et al,[18] an asymptomatic, "mild-to-moderate" rise of serum total AMY or lipase concentrations was found in 60% of HIV+ patients at any time of their clinical history, but this rate dropped to 14% when a threshold of more than 2 times the URLs for defining an enzyme elevation was applied. The lack of agreement among studies in defining biochemical pancreatic abnormalities made, therefore, results hardly comparable. Regrettably, the same was also true for the detection of overt acute pancreatitis.[5,8,10]

Our results in a large cohort of patients using a URL derived with a very robust protocol indicate the presence of pancreatic hyperamylasemia in approximately 14% of HIV+ patients. An important observation in our study is that neither HIV infection nor HAART increased the rate of pancreatic hyperamylasemia in comparison with control groups. It is, however, important to note that our HIV– control group mainly included patients with liver disease, a condition supposed to increase serum P-AMY too.[11,27] In our HIV+ population, pancreatic hyperamylasemia was generally mild and in most patients far lower than the best decision limit for acute pancreatitis (ie, >3 times the URL). Moreover, in almost half of hyperamylasemic patients, lipase concentrations were normal, indicating a nonpancreatic origin of their P-AMY increase. Only three HIV+ patients with pancreatic hyperamylasemia reaching a level compatible with overt pancreatitis showed a frank pancreatic disease, with one having a discharge diagnosis of acute disease (Table 3). Our results partly oppose the conclusion of a recently published review assessing the role of HAART in the development of episodes of acute pancreatitis, stating that "drug-induced pancreatitis is frequently not acknowledged, because mild cases, with significant (but not critical) increases in amylase and lipase levels, may go unnoticed, in addition to the possible dissociation at the time of exposure to the drug and the development of acute pancreatitis."[28]

Another interesting observation is that in our HIV+ population, we found a total of eight cases of MA; this

Table 1
Frequency of Pancreatic Hyperamylasemia in the Studied Population

Group	No. of Patients	Patients With P-AMY >URL, No. (%)	*P* Value	Patients With P-AMY >URL and Lipase ≤URL Relative to all Hyperamylasemic Patients, No. (%)
Total	1,548	221 (14.3)		106 (48.0)
HIV negative	92	14 (15.2)	.91	6 (42.9)
HIV positive	1,456	207 (14.2)		100 (48.3)
No therapy	123	11 (8.9)	.11	4 (36.4)
HAART	1,333	196 (14.7)		96 (49.0)

HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; P-AMY, serum pancreatic amylase; URL, upper reference limit.

Table 2
Characterization of Patients With Macroamylasemia

Age, y	Sex	Category	Supernatant P-AMY Residual Activity, %	P-AMY, U/L	Lipase, U/L	P-AMY/Lipase Ratio
47	Male	HIV+ on HAART	11	103	22	4.68
46	Male	HIV+ on HAART	15	62	41	1.51
67	Female	HIV+ on HAART	18	110	31	3.55
62	Male	HIV+ on HAART	18	137	27	5.07
48	Male	HIV+ on HAART	20	719	41	17.54
57	Male	HIV+ on HAART	33	136	16	8.50
41	Male	HIV+ no therapy	34	59	64	0.92
36	Male	HIV+ on HAART	34	413	31	13.32

HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; P-AMY, serum pancreatic amylase.

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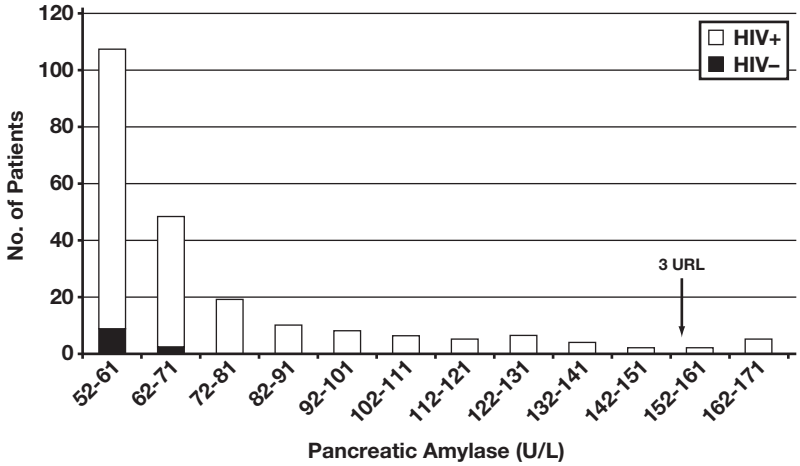

Figure 1 Frequency distribution of pancreatic hyperamylasemia in the studied population. URL, upper reference limit.

Table 3
Characterization of Patients With Marked Pancreatic Hyperamylasemia, Defined as Concentrations More Than Three Times the Upper Reference Limit

Age, y	Category[a]	P-AMY, U/L	Lipase, U/L	P-AMY/ Lipase Ratio	Serum Creatinine, mg/dL	Diagnosis
40	HIV+ on HAART	160	267	0.60	0.88	Acute pancreatitis
53	HIV+ on HAART	161	286	0.56	0.73	Chronic pancreatitis
50	HIV–	184	114	1.61	1.72	Chronic pancreatitis
34	HIV+ no therapy	214	258	0.83	0.46	Chronic pancreatic hyperenzymemia of undefined origin
49	HIV+ on HAART	270	469	0.58	0.70	Chronic pancreatitis
36	HIV+ on HAART	413	31	13.32	0.87	Macroamylasemia
48	HIV+ on HAART	719	41	17.54	0.99	Macroamylasemia

HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; P-AMY, serum pancreatic amylase.
[a]All male patients.

corresponds to an MA incidence of 0.55%, which is not so different from the 1% figure reported in the general population.[11] Although it is well known that MA may be also detected in the presence of normal serum AMY values,[17] and therefore our finding in hyperamylasemic patients may be underestimated, it is clear that we were unable to confirm the high incidence of MA in the HIV+ population (13%) found by Foo and Konecny[9] in their pioneering study. We would emphasize that the PEG precipitation method used in our study may have some limitations, mainly when serum globulins are present in excess, as in hypergammaglobulinemic HIV+.[29] However, in these conditions, PEG precipitation may produce false-positive macroenzyme results, so that a spurious increase in MA incidence as method interference theoretically would be expected. On the other hand, confirmatory techniques, such as electrophoretic separation or gel filtration chromatography, are too cumbersome to be applied in large-scale studies such as ours, not to mention that some authors did not recommend electrophoresis for use in the detection of MA because of the large variability in migration patterns.[30]

We are aware that our study has a major limitation represented by the relatively small number of patients in the control groups (HIV– and untreated HIV+). This can make it difficult to compare the prevalence of pancreatic hyperamylasemia. However, the evaluated population represents the real-life consecutive patients referred to our infectious disease clinic in a time period of 6 months.

In conclusion, in our study, both HIV infection per se and HAART did not increase the frequency of P-AMY elevation. Furthermore, when present, pancreatic hyperamylasemia was generally mild, and frank pancreatic disease, especially acute pancreatitis, was relatively infrequent in this clinical setting. As a consequence, the use of P-AMY measurement as a screening test for detecting pancreatic injury in asymptomatic HIV+ individuals is not recommended. Further studies are needed to determine factors univocally associated with the risk of pancreatic injury development to

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identify the subset of HIV+ patients who should selectively be evaluated and monitored using pancreatic enzymes.

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Conflict of interest: none declared.

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