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Pancreatic isoamylase as a routine test

by Gunnar Skude, Department of Clinical Chemistry, County Hospital, Halmstad, Sweden
gunnar.skude@lthalland.se

SUMMARY

α-Amylase (1,4 -α-D-glucan-4-glucanohydrolase E. C. 3.2.1.1.) catalyses the hydrolysis of starch, glycogen and related poly- and oligosaccharides. The end products formed are maltose, short chain dextrins and some glucose. In man large amounts of amylases are secreted into the digestive tract by the salivary and pancreatic glands, minimal amounts also being produced in other tissues. Recently, techniques for specific determination of pancreatic isoamylase activity in plasma have been developed. Routine use of such techniques will increase the clinical value of amylase determination.

In this article

- "In order to give the clinicians best possible information of their requested amylase determinations it can be concluded that the test should be performed on plasma/ serum and not on urine"
- "Amylase determinations are performed to disclose and monitor diseases of the pancreatic gland and consequently they should be performed as pancreatic isoamylase determinations"

General survey of methods

Since Wohlgemuth introduced his semiquantitative method for determination of "diastase" in urine as a test of pancreatic disease during the first decade of the last century, amylase determination has been a valuable tool in practical medicine. The method of Wohlgemuth was widely used for determining amylase activity during the following half century, after which it was replaced by more sophisticated techniques. These new methods were either amyloclastic, in which the decrease in colour intensity in the reaction between starch and iodine was monitored and taken as an index of starch digestion, or saccharogenic, i. e. the amount of liberated glucose, maltose or isomaltose was determined. Other principles, such as the use of chromogenic substrates for the detection of amylase activity were also developed and are still used. The latter methods are based on the principle that the amylolytic activity of the enzyme liberats small solubl e fragments from an insoluble dye-starch polymer into the reaction solution; the amylase activity is thus proportional to the staining intensity.

During the last decades methods utilizing defined maltooligosaccharides (G3 to G7) as substrates in combination with sacharogenic or chromogenic determination have been introduced and largely replaced previously used methods. In total about 250 methods for amylase determination have been described. This high number might indicate that we still have not got a satisfactory technique for determination of amylase activity. Recently an IFCC working group published recommendations for amylase de-

termination (1). The recommended method is based on the use of nitrophenylmaltoheptaoside as substrate and the enzyme glucosidase to liberate 4-nitrophenolate from the degradation products. To prevent glucosidase degradation at the non-reducing end of the substrate the terminal glucose is blocked by an ethylidene bridge. Thus , this method modification is called EPS (ethylidene protected substrate).

The standardization of the IFCC method is based on the amount of liberated 4-nitrophenol using the molar absorption coefficient which, however, is influenced by many factors such as protein concentration, temperature, pH, buffer components and chloride concentration. Thus, calibration of the catalytic activity must be based on an empirically determined, and generally accepted, molar absorption coefficient. The IFCC working group on calibrators in clinical enzymology have in progress a multi enzyme reference material including amylase.

Isoamylases

Total amylase activity of a blood or a urine sample may be useful in the diagnosis of a lesion of an organ producing amylase. However, more than one organ can produce amylase, thus decreased amylase release from one organ may be masked by the release of amylase from another organ. In other cases the origin of an increased plasma amylase activity may be obscure.

Since the late fifties it has been known that many enzymes exist in multiple molecular forms which can be separated from each other due to differences in their physicochemical properties. Many of these isoenzymes are more or less tissue specific. Hence, changes of the normal plasma (or serum) isoenzyme pattern may be diagnostically useful even when the total enzyme activity is non-informative.

Total amylase activity of normal plasma originates from the salivary and the pancreatic glands; the contribution to the plasma activity from the two sources being roughly equal. Minor amounts of amylase are also produced in some organs of the female genital tract but their contribution to the plasma activity is negligible except in some cases of genital malignancies. Increased plasma activity of amylase might also be seen in patients with tumours of the respiratory tract. In both of these cases the amylases produced are of the salivary isoenzyme type.

Macroamylasemia is a rare condition seen in about 0.1-0.2 per cent of patient samples. It is characterized by moderately increased plasma activity of amylase while the urine amylase excretion is reduced. The increased amylase levels are due to a complex formation between amylase and another protein, usually IgA. The amylase moiety of the complex can be of either salivary or pancreatic isoamylase type. Due to the high molecular mass, the renal elimination of the complex is reduced. In spite of the abnormally increased amylase level in plasma the condition has no clinical impli-

IFCC Technical Secretariat,
30 Rue Lionnois,
F-54000 Nancy,
France

Phone: +33 3 83 35 26 16
Fax: +33 3 83 32 13 22
Email: Chantal.Thirion@ifccts.u

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cation.

So far isoamylase determination has not been widely used in clinical practice although the activity the salivary and pancreatic groups of isoamylases can be separately determined.

Among the commonly used methods for isoenzyme separation and subsequent activity determination the following can be mentioned; electrophoresis, temperature inhibition, or activity determination using isoenzyme specific substrates, at various pH-levels, in the presence of an inhibitor, or after immunologic activity inhibition. As many of these techniques are laborious and consequently also expensive, isoamylase determination in routine clinical practice has not been widely used until now. Recently, however, commercially available procedures for the specific determination of pancreatic isoamylases in plasma/serum using routine clinical chemistry analysers have been developed. The activity of the salivary isoamylase is inhibited by two different monoclonal antibodies having no effect on the activity of the pancreatic isoamylases (2, 3). After subsequent incubation with the above mentioned maltooligosaccharide the pancreatic isoamylase activity can be specifically disclosed using the IFCC method. This technique shows excellent correlation with other earlier used methods (4).

Pancreatic isoamylase versus total amylase

From a clinical point of view it is almost always the activity of pancreatic isoamylase that is of interest to evaluate. The new techniques make it possible to substitute specific pancreatic isoamylase determination for total amylase determination in order to avoid disturbing effects due to the individual variation of the salivary isoamylase. Thus, the informative value of an amylase determination performed is increased when pancreatic isoamylase is analysed instead of total amylase.

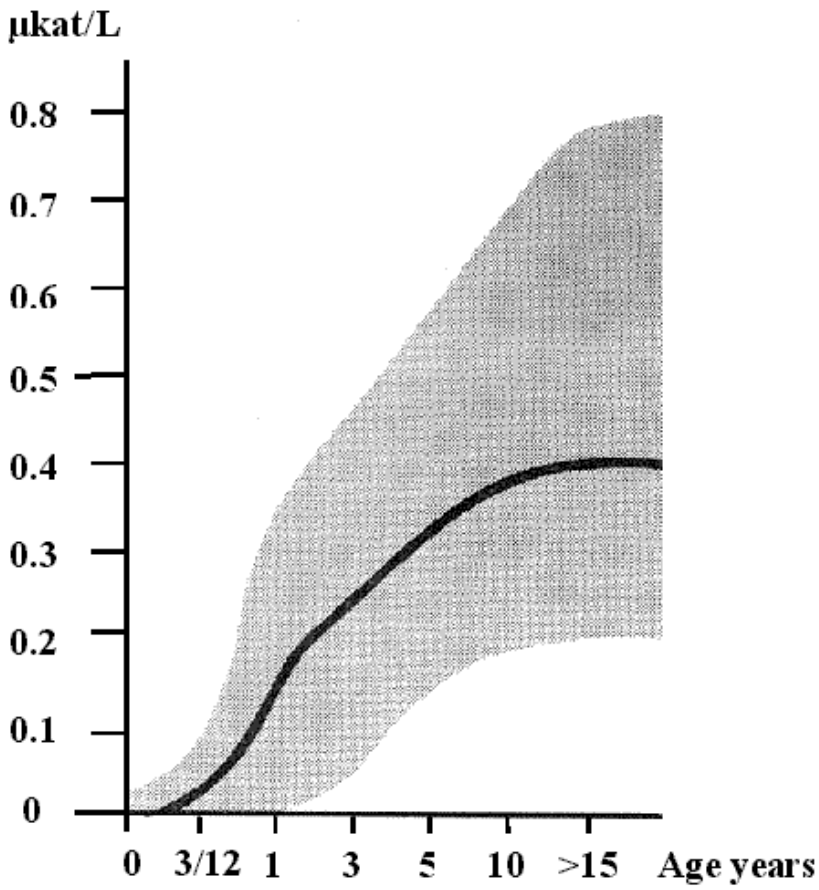

Figure 1

At birth the activity of the pancreatic group of isoenzymes in plasma is extremely low; the majority of *normal* children below 3 months of age have no detectable pancreatic plasma isoamylase activity (Figure 1). After the first few months of life the activity rises slowly to reach the adult level at the age of 10 to 15 years (5). However, below the age of one a complete absence of pancreatic isoamylase in plasma can be regarded as a normal finding. The activity of pancreatic isoamylase in plasma of older children and adults can be considered as normally distributed. The activity does not show any gender related difference and there is no significant diurnal variation, nor is there in normal persons any variation due to food ingestion.

In *acute pancreatitis* the hyperamylasemia is characterised by an increased activity in plasma of pancreatic isoamylase. As the salivary and pancreatic isoamylases normally contribute about equally to the total plasma activity the increase in relation to the reference value is doubled for pancreatic isoamylase compared to total amylase. The sensitivity of pancreatic isoamylase determination in plasma/serum is significantly higher than that of total amylasein the diagnosis of acute pancreatitis (2). In acute pancreatitis the activity of the pancreatic isoamylase varies within wide limits, from within the reference range to more than 50 times the upper reference limit, according to the functional state of the pancreas, the severity, and cause of the inflammation.

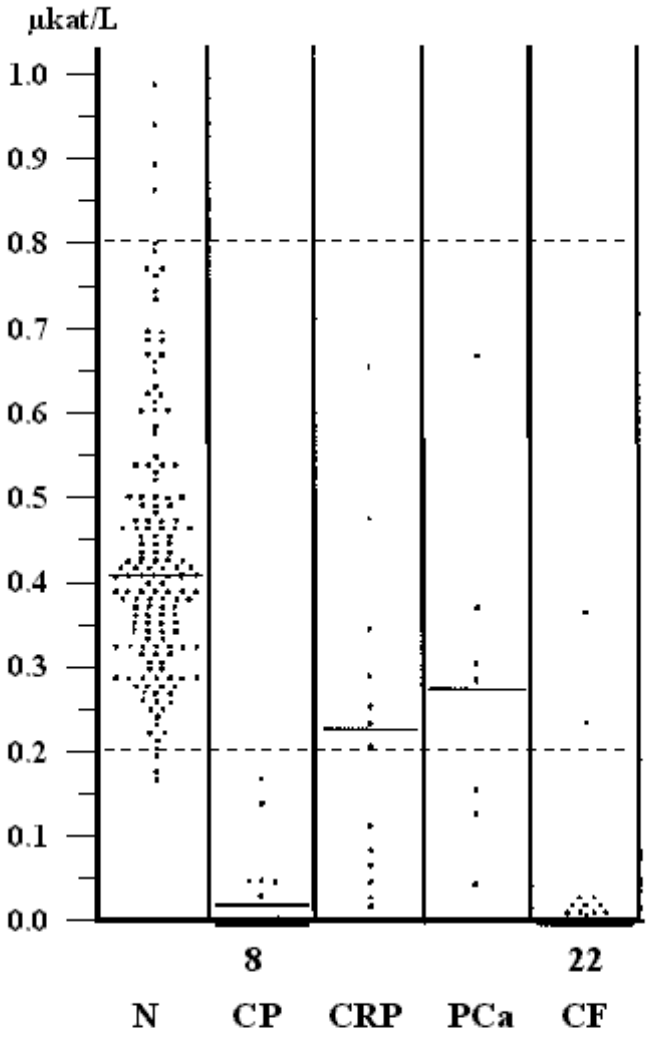

Figure 2

During the following 4 -10 days the pancreatic isoamylase activity gradually decreases to normal level. The half-time of pancreatic isoamylase activity in plasma is about 12 hours.

In *chronic pancreatitis* with exocrine pancreatic insufficiency the total amylase activity in plasma is

as a rule normal although the pancreatic isoamylase activity is greatly decreased. This is due to the fact that the salivary isoamylase activity often is increased in plasma in these patients. In a small study comprising 15 pati ent s with advanced chronic pancreatitis without obstructive symptoms i.e. pain or jaundice at the time of investigation but having steatorrhea, more than half had no detectable pancreatic isoamylase activity in plasma (Figure 2). All patients had subnormal pancreatic isoamylase activities in plasma although all but one had normal total amylase activity (6). Corr esponding results have been reported from other groups independent of isoamylase method used (7, 8, 9).

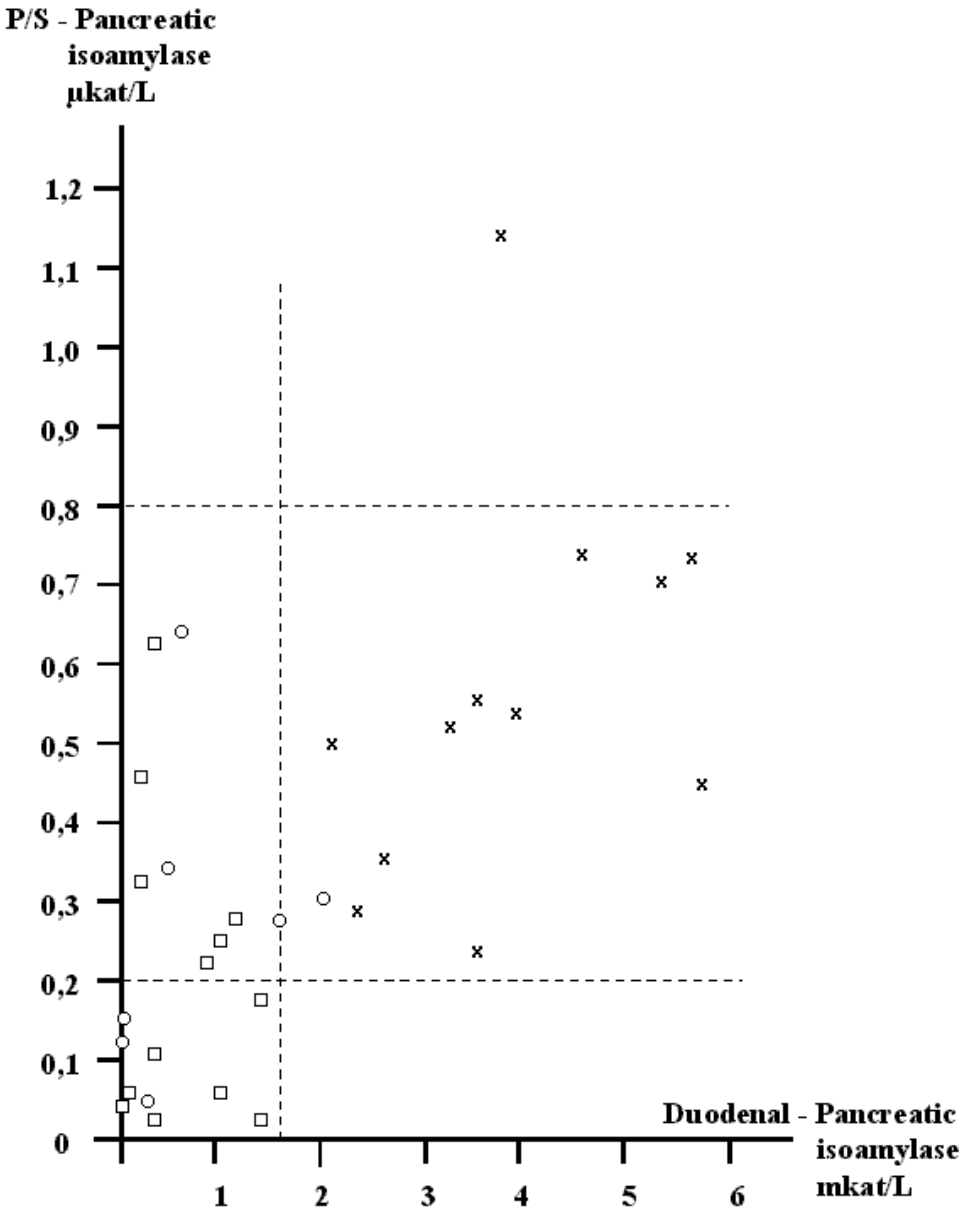

Figure 3

Patients with abnormally decreased pancreatic isoamylase activity in their plasma also have reduced pancreatic isoamylase activity in their doudenal aspirates obtained in connection with a test meal for evaluation of the pancreatic function (Figure 3)(10, 7, 8, 9). Thus, a decreased pancreatic isoamylase activity in plasma identifies patients with *exocrine pancreatic insufficiency* and makes intubation tests or other complicated tests for pancreatic function unnecessary. If, however, the pancreatic isoamylase activity in plasma is normal a reduced pancreatic function cannot be excluded.

In patients with *chronic relapsing pancreatitis* the total plasma amylase activity is often normal al-

though the pancreatic isoamylase activity is more or less decreased in 50-65 per cent of the patients (Figure 2 and 3). In some patients, mainly those having obstructions in their duct system or pancreatic cysts, increased plasma activities of pancreatic isoamylase can be seen (10, 7, 8, 9).

The diagnostic value of pancreatic isoamylase determination in plasma as compared to total amylase activity has been studied in 213 patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) because of suspected pancreatic disease (11). In patients with radiographic evidence of pancreatitis less than 1/3 had abnormal, as a

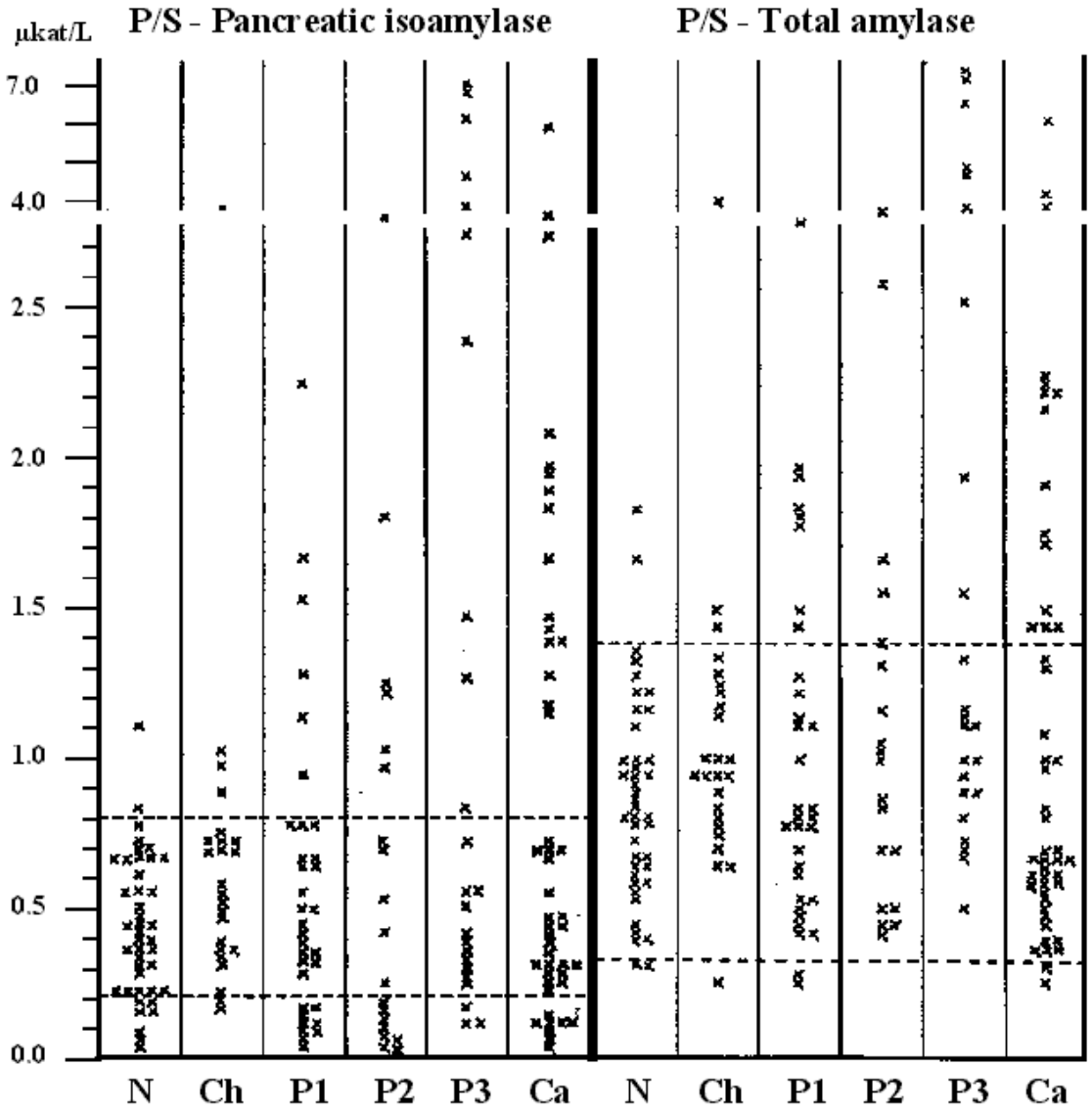

Figure 1

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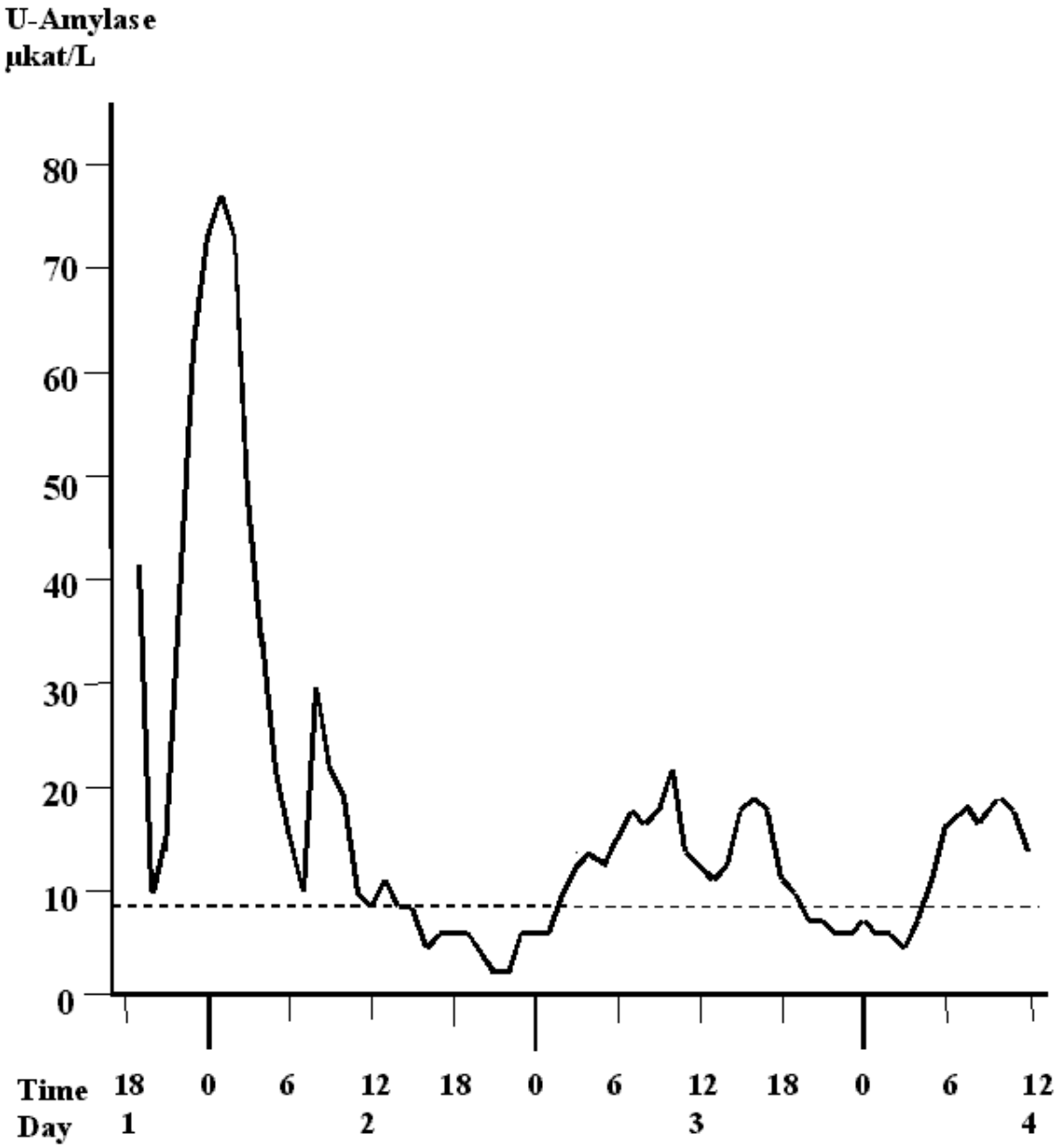

Figure 5

rule increased, total plasma amylase activity (Figure 4). Determination of the pancreatic isoamylase activity, however, disclosed abnormal activities in 45 per cent of 33 patients with mild-moderate pancreatitis, in 74 per cent of 23 cases with advanced pancreatitis with calculi, and in 63 per cent of 27 p atients with advanced pancreatitis with signs of obstructions in the duct system (Figure 4). In the first

two patient groups the activities were mainly reduced but in the patients with signs of obstruction the activities were mainly increased. The corresponding percentage of abnormal total amylase activity was 30, 22, and 33 per cent, respectivily. From Figure 4 it is also evident that a number of patients have no radiographic evidence of pancreatic disease although the secretory capacity of the pancreas is decreased as disclosed by a low pancreatic isoamylase activity in their plasma/serum.

In patients with *pancreatic carcinoma* the total amylase activity in plasma was found to be abnormal in about 30 per cent of the cases; whereas the pancreatic isoamylase activity was abnormal almost twice as often, in 53 per cent (10). I ncreased as well as decreased plasma activities were seen in the cancer patients (Figure 4).

About 70 per cent of patients with *cystic fibrosis* have no detectable pancreatic isoamylase activity in plasma. About 20 per cent have d ecreased activity and just 10 per cent or less have a normal plasma activity of pancreatic isoamylase although the total amylase activity is normal (Figure 2)(12, 13, 5).

Transient postoperative hyperamylasemia has repeatedly been reported (14, 15, 16). The hyperamylasemia, occurring in 10 per cent or more can be due to either salivary or pancreatic isoamylases. Elevated salivary isoamylase activity is seen after all types of operations while increased pancreatic isoamylase activities was limited to surgery of the pancreas itself or close to it. Similarly hyperamylasemia is seen fo l-lowing *duodenoscopy* and *ERCP* (17). In about 10 per cent the activity of the salivary isoamylases increases more than 1.3 times the initial activity, the increase can be up to 10-fold. Routine use of pancreatic isoamylase determination instead of total amylase would of course result in missing this information which, however, must be considered as unimportant and misleading from the clinician´s point of view.

Due to reduced glomerular filtration pancreatic hyperamylasemia may occur in *renal insufficiency* without clinical signs or symptoms of pancreatic disease (18). There is no influence of *oral amylase substitution* on the pancreatic isoamylase activity in serum.

In order to evaluate the value of *routine isoamylase determination,* a trial was started 1980-81 at a county hospital serving about 125 000 persons (19). During a six-month period isoamylase determinations were substituted for all total amylase determinations using a selective inhibitor produced from wheat to differentiate between the salivary and pancreatic types of isoamylases (20). During this period almost 2 800 patient plasma or se rum samples were analysed. About 550 of the samples had increa se d total amylase activity which in 17 per cent was find to be due to increased increased activity of salivary isoamylase. About 1 800 of the samples had normal total

amylase activity although 12 twelve per cent of these samples had increased and 8 per cent had decreased pancreatic isoamylase activity. Of the 450 samples having reduced total activity 49 per cent had normal pancreatic isoamylase activity. Thus, total amylase determination in plasma/serum gave false information concerning the state of the pancreas in 24 per cent of the samples, a figure which was in good agreement with the diagnoses obtained from the patient records.

The clinical value of routinly performed isoamylase determination has also been pointed out by other groups. Thus, one group found hyperamylasemia in 139 of 2350 consecutive samples analysed at a university hospital in Japan (21). In over half of the sera the hypeamylasemis was caused by increased salivary isoamylase activity.

Thus, it must be concluded that determination of the pancreatic isoamylase activity of plasma is superior to determination of total amylase activity to disclose pancreatic disease.

Plasma versus Urine

When Wohlgemuth introduced his method for determination of "diastase", urine was the natural system to be used. A urine sample is easy to obtain and handle at the laboratory. Furthermore it has been stated that in monitoring patients with acute pancreatitis urine is superior to plasma/serum as the amylase activity remains elevated for a longer period of time in this system as compared to plasma. Our studies showed that the pancreatic isoamylase activity of urine in 45 per cent of cases normalized the same day; in 50 per cent one day before and in 5 per cent one day after. Taking care to other laboratory parameters and the clinical status of the patient this difference is most likely clinically insignificant

Due to variation of the diuresis the reference ranges for analytes in urine are much wider than the corresponding ranges in plasma. For pancreatic isoamylase activity the span of the reference range amounts to 3 times the lower reference limit in plasma as compared to 21 times in urine. When relating the amylase excretion to creatinine this figure is reduced to about 4.5 but requires an additional analysis. The result is still not as good as in plasma. The amylase activity in urine varies considerably within very short periods of time (Figure 5). Thus, it must be concluded that urine has some severe disadvantages as compared to plasma/serum as system for determination of amylase activity. Acute pancreatitis is a severe disease with a very high mortality, and if such a disease is suspected enzyme activity determination in plasma/serum is a prerequisite.

State of the Art in Sweden today

During the last decade there has been a shift in Sweden from the determination of total amylase to pancreatic isoamylase and to the exclusive use of plasma as a system in-

stead of urine. This change is based on the findings referred to above. At the beginning of the new millennium 67 of the 81 hospital laboratories taking part in Swedish external control programme, EQUALIS, analysed amylase as pancreatic isoamylase. Of the remaining 14 laboratories, 10 were forced to determine total amylase as they use instruments based on dry chemistry which at present cannot easily be used for specific pancreatic isoamylase determination. Just four of the laboratories still perform total amylase determinations by their own choice.

When EQUALIS started the external quality programme in 1993 the coefficient of variation (CV) for total amylase determinaton was about 38 per cent. The few laboratories performing pancreatic isoamylase at that time exhibitied a CV of about 25 per cent. These figures remained constant for the following five years. It was then decided to harmonise the amylase determinations in Sweden. Electrophoretic analysis of existing commercial calibrators revealed that they contained a mixture of human isoenzymes as well as in some cases non-human amylases. In some cases also the matrix was considered dubious and it was concluded that the ideal calibrator for amylase activity determination should be based on human plasma. In addition the amylase should be exclusievly of human pancreatic origin.

Based on these considerations an amylase calibrator was prepared (6). As matrix plasma was used from individuals lacking salivary isoamylase in their plasma due to genetic factors. As, however, the pancreatic isoamylase activity in these sera was much to low for a useful calibrator, amylase had to be added. The amylase added was solely the main fraction of purified human pancreatic isoamylase from individuals showing the most common inheritied isoamylase type (Figure 6). In this way matrix effects due to variation in equipment and reagents were considered to be abolished. The activity of the calibrator was determined in two laboratories independently. The calibrator was distributed to various laboratories together with a control prepared in the same way but also containing the main salivary isoamylase fraction.

After the introduction of the new calibrator the CV of the 67 laboratories performing pancreatic isoamylase determination has decreased from about 25 per cent to 6 per cent. For the laboratories performing total amylase determination the CV has decreased from 38 to about 20 per cent. This figure is still high but it may be that all of these laboratories have not yet introduced the new calibrator which is recommended also for total amylase determination.

The introduction of the new amylase calibrator and harmonisation of pancreatic isoamylase determinations made it also feasable to introduce identical reference ranges in most of the hospitals throughout our country.

Conclusions

In order to give the clinicians best possible information of their requested amylase determinations it can be concluded that the test should be performed on plasma/serum and not on urine. Amylase determinations are performed to disclose and monitor diseases of the pancreatic gland and consequently they should be performed as pancreatic isoamylase determinations. To-day techniques for simple and cheap pancreatic isoamylase determination exist and thus this test can be performed on most of our routine clinical chemistry analysers. By the use of a pure human calibrator containing only pancreatic isoamylase, matrix effects are abolished and harmonisation of the amylase determinations within a geographic area is easily obtained and common reference ranges can be adopted. Thus, the risk of misjudging patients is reduced when doctors or patients move from one area to another.

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IFCC Technical Secretariat,
30 Rue Lionnois,
F-54000 Nancy,
France

Phone: +33 3 83 35 26 16
Fax: +33 3 83 32 13 22
Email: Chantal.Thirion@ifccts.u

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