Practical Laboratory Medicine 8 (2017) 65–69

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Distribution of serum concentrations reported for macroenzyme aspartate aminotransferase (macro-AST)

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ARTICLE INFO

Keywords:
Macroenzyme
Aspartate amino transferase
Immunoglobulins
Mathematical model

ABSTRACT

Background: The presence of macroenzyme (M) is often the explanation of an isolated elevation of aspartate aminotransferase (AST). Where M is identified, it is reasonable for the clinician to ask where an individual patient's result fits in with known concentrations of M. In this context, we conducted a survey of literature to examine the distribution of reported serum concentrations of macro-AST. We also analyzed the distribution data to examine whether elevations were consistent with simple alteration of circulatory half-life ($t_{1/2}$) of M relative to normal AST.

Methods: Distributions of M were compiled from the literature. These distributions were compared to predictions based on fixed changes in $t_{1/2}$ applied to the reference interval for AST.

Results: There was a bimodal distribution of literature values for M ($n = 51$), comprised roughly of populations A ($M < 200$ U/L; 60% of total) and B ($M > 200$ U/L; 40% of total). The two distributions were reasonably well characterized by a simple projection to the right of the reference interval for AST according to increased $t_{1/2}$ (A: $t_{1/2} = 3.3$ days; B: $t_{1/2} = 19.8$ days) relative to AST ($t_{1/2} = 0.7$ days).

Conclusions: Knowledge of distributions for M may be useful in discussion with clinicians regarding significance of M for individual patients. Distributions for M were consistent with the simplest explanation for elevated AST due strictly to an extended circulatory lifetime for M. Caveats to analysis, however, include selection within literature data mainly for patients with various co-morbidities.

1. Introduction

The presence of a macroenzyme (complexes of an enzyme, either as multimers, multi-protein complexes, immunoglobulin complexes) are well-known as a potential cause of isolated elevation of individual enzymes in patients having no related clinical symptoms [1–3]. The elevations are generally assumed to reflect an extended circulatory lifetime of the macroenzyme relative to the non-complexed enzyme [2]. Our laboratory is occasionally asked to evaluate isolated enzyme elevations for the presence of macroenzymes. There exist multiple methods for macroenzyme detection [1,4–8]. In our laboratory, initial evaluation is based simply on lability of sample concentration to polyethylene glycol (PEG) precipitation [9,10].

We recently evaluated a case of unexplained elevation of AST for the presence of macro-AST. The patient was a 46 year old Caucasian male with an isolated, persistent AST elevation ranging from 156 to 428 U/L over the prior year's repeated testing (reference range: 7–42 U/L). His ALT was continuously in the range of 11–18 U/L (reference range: 9–46 U/L). His alkaline phosphatase and total bilirubin levels were also within reference range limits. Hepatic imaging revealed a normal liver morphology with no evidence of steatosis, and work-up for all etiologies of transaminitis, including viral, autoimmune and genetic liver disorders,

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http://dx.doi.org/10.1016/j.plabm.2017.05.003
Received 1 January 2017; Received in revised form 12 April 2017; Accepted 15 May 2017
Available online 17 May 2017

was unremarkable. Diagnostic testing for thyroid disease, muscle disorders, hemolysis and celiac disease was also negative. There was no evidence of drug-induced liver injury, as his only medications included Nasonex nasal suspension and ProAir metered-dose inhaler for seasonal allergies and asthma. Moreover, the patient denied any history of alcohol, illicit drug use, over-the-counter or herbal medications. There was also no significant family history of note. “Macro-AST” was the suspected clinical diagnosis. By PEG pretreatment, the elevation was found to be consistent with macro-AST. Macro-AST is commonly due to association with IgG [1], but can also be due to association with IgA or IgM [11–13].

The ordering physician asked whether, despite this finding, one could still rule out a circumstance of overproduction of AST. Experimentally, methods such as electrophoresis and immunofixation could detect whether the measurement of elevated AST with detection of macroenzyme might also include an elevated free fraction. Even without an elevated free fraction, however, it is certainly theoretically possible for an elevated concentration to reflect both abnormal production in addition to prolonged lifetime of the macroenzyme. Thus, one approach to the question is to determine where a given patient's results stand with respect to the range of reported values for macro-AST.

In this context, we performed a literature review for reported concentrations of macro-AST. Additionally, we also examined the distribution for reported values for macro-AST from a mass balance perspective, to ask whether the results range for macro-AST was compatible with a simple shift of the reference range for AST due solely to an altered circulatory lifetime for different forms of macro-AST.

2. Methods

Primary data were macro-AST (M) concentrations compiled from literature reports identified through PubMed (e.g., searched using “macroenzymes AND aspartate aminotransferase [tw]”) and published prior to 2017. Data analysis and statistical calculations were conducted using Excel.

3. Results

3.1. Distributions of macro-AST concentrations

Results were 51 concentrations of macro-AST ([M]) reported in the literature [5,8,11–31], excluding 1 case involving fluctuation of AST measurements in presence of a complicated pregnancy [32], and 1 case involving association of increasing AST in the presence of myeloma [33]. The distribution of results is shown in Fig. 1. By visual inspection, the distribution was comprised of two generally distinct populations, A and B: A. [M] < 200 U/L, 60% of total (n = 31), 62–189 U/L, average 117 U/L, median 113 U/L; and B. [M] > 200 U/L (n = 20), 40% of total, range 233–1150 U/L, average 490 U/L, median 431 U/L. As will be shown below, this apparent division between populations at approximately 200 U/L is consistent with a physiological explanation of two sets of M having distinct circulatory half-lives ($t_{1/2}$'s). The patient's highest AST result (428 U/L) was close to the median for group B.

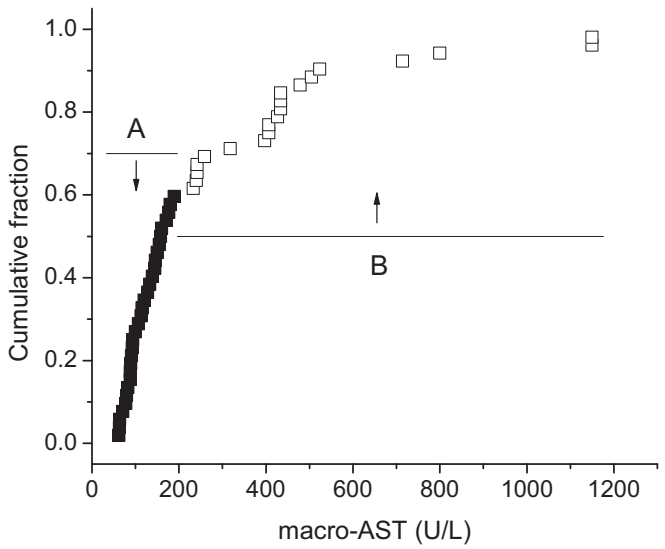

Fig. 1. Distribution of literature values for [M] (cumulative fraction vs. [M]). Data were divided into groups A (< 200 U/L, open points; lower 60% of results) and B (> 200 U/L, closed points; upper 40% of results). Groups A and B were compared to central 95% ranges of normal distributions for M (lines) calculated from the reference interval for E (7–42 U/L) when assuming lesser $t_{1/2}$: line A. $t_{1/2}$ = 3.3 d (k’ = 0.21/d); range: 33–196 U/L; line B. $t_{1/2}$ = 19.8 d (k’ = 0.035/d); range: 196–1176 U/L.

3.2. Model analysis of distributions of macro-AST

Distributions for M were analyzed with respect to predictions based on the assumption that elevated M is due simply to an alteration of the circulatory half-life ($t_{1/2}$) of M relative to normal AST. Designating a normal AST as enzyme (E), we assumed that [E] in plasma usually represents a steady-state (d[E]/dt = 0), balancing the rate of appearance in plasma (ki, where ki ≡ concentration/time) and the rate of elimination (ke, where ke ≡ concentration/time). The rate of elimination was assumed to be first order with respect to enzyme concentration, ke = k[E] (where k ≡ 1/time). Thus, in steady-state, ki = k [E]. For macroenzyme M, altered ke was assumed to reflect a substantial change in the rate constant k to a lesser value, k'. Assuming that ki is unaltered in conditions in which M is present (i.e., there is no overproduction of AST from which M is formed), then ki = k' [M]. Thus, [M] should be related to an otherwise normal enzyme concentration [E] according to the equation k [E] = k' [M], or, [M]/[E] = k/k'. With these assumptions, the distribution for M should represent a simple shift in the reference range for E (the reference interval for [AST]) by the factor k/k'. For normal AST (E), k was taken from the literature: $t_{1/2}$ = 17 h (0.71 days); k = 0.98/day [34], where k for a first order process is given by k = -ln(0.5)/($t_{1/2}$) (that is, k is the value for which exp(-k $t_{1/2}$) = 0.5).

By the mass balance analysis, M was predicted to be a distribution that was shifted upward in concentration from the reference interval distribution for E by the factor k/k'. The reference interval for E (7–42 U/L) was assumed to be non-parametric, that is, representing the boundaries of the central 95% of results from among normal subjects, as it is known that the reference interval distribution for AST is positively skewed relative to a normal (Gaussian) distribution (mean > median) [35,36]. Example shifts of the boundaries of the AST reference interval according to a factor k/k' are shown in Fig. 1 for datasets A and B.

For group A, a shift of the reference range for AST using k' = 0.21/d is shown, for which $t_{1/2}$ = 3.3 days. For group B, a shift of the reference range using k' = 0.035/d is shown, for which $t_{1/2}$ = 19.8 days. The range of the distributions show that a simple shift of the AST reference interval by an altered k' is a plausible interpretation of the data for both datasets A and B. The analysis of half-lives is only to say that the data might have this physiological explanation, but characterization of the data in this manner is by no means evidence that such an explanation is correct.

The populations for datasets A and B having different $t_{1/2}$'s might plausibly be presumed to be due to M derived either from IgA or IgM (shorter half-life; dataset A), or from IgG complexes (longer half-life; dataset B). In context of the model calculations, dataset A showed an apparent half-life that was reduced by approximately 50% relative to that of IgA or IgM ($t_{1/2}$ = ~ 6 days, for which k' = 0.116/day), whereas for dataset B, the apparent half-life was essentially identical to that for IgG ($t_{1/2}$ = ~ 20 days, for which k' = 0.035/day) [37].

4. Discussion

Macroenzymes are most often documented in the context of an investigation of elevation of enzyme in the absence of elevation of other physiologically associated enzymes (an isolated elevation). Arguably, the identification of a macroenzyme in the absence of elevation of other associated enzymes might be regarded as sufficient clinical information regarding a benign process. In certain cases, however, there may be very close scrutiny given to any abnormal findings (e.g., transplant and pre-transplant patients, or subjects who are candidates for a clinical trial). The data compilation in this study can serve as a reference for the added information of whether a given elevation is within the observed range of elevations for M.

A finding from the study was that the literature data for the distributions of concentrations of M were broadly compatible with the premise that the elevated concentrations observed are due strictly to an increased $t_{1/2}$, unaccompanied by any alteration in the rate of appearance of enzyme into circulation. This added information may be useful to the clinician, or reassuring to the patient, that the abnormal results reflect only a benign process. Electrophoresis and immunofixation would be recommended analyses for followup were further characterization of M required, especially to determine whether in fact the elevated enzyme concentration was due to a predominant macroenzyme fraction, or if it instead included a significant fraction of non-macro, native enzyme.

Multiple methods exist for detection and characterization of macroenzymes [1,4–8]. However, clinical case reports of macro-AST only rarely report investigation or identification of an interacting immunoglobulin. Among 49 examples of M obtained from the literature, immunoglobulin identification was reported for only six, including one IgM and including no identifications of IgA (Table 1). Note that M may not necessarily represent a homogeneous population of macro-AST, but may instead represent a heterogeneous combination of E and M. Additionally, it is possible for M to be a heterogeneous combination of immune complexes with different immunoglobulin types [11]. In our description of Fig. 1, separation of M into two supposed classes was initially only a rough interpretation based on the appearance of a bimodal distribution of results. Characterization of the two classes as classes of

Table 1
Macro-AST examples having immunoglobulin identification.

Case	[M] (U/L)	Class	Reference
1	146	IgG	Briani et al. [22]
2	171	IgM	Matsuda et al. [13]
3	188	IgG	Collins et al. [21]
4	233	IgG	Fortunato et al. [18]
5	259	IgG	Matama et al. [15]
6	434	IgG	Vajro et al. [14]

immunoglobulins having distinct $t_{1/2}$'s is physiologically plausible but speculative. To our knowledge, there has not been an investigation concerning whether there is a relationship between oligomeric states of immunoglobulins to the level of macroenzyme observed, as in, for example, whether pentameric IgM correlates with a higher or lower concentration of macroenzyme.

Caveats to the data analysis include the fact that there may be a variety of types of selection bias among patients reported in the literature. Most patient reports are cases in which macro-AST was an incidental finding in the context of some other primary diagnosis that is seemingly unrelated. A recent report identified an unusual case in which IgA-complexed macro-AST was associated with an IgA monoclonal gammopathy [38]. In general, however, etiologies and the potential effects of comorbidities on circulatory lifetimes of macro-AST are unknown. An additional caveat to the analysis is that there are likely to have been many different methods used for AST measurement in the studies cited. For instance, the inclusion or exclusion of pyridoxal phosphate in AST activity measurements is a known variable across different assay platforms [32], and thus the commutability of results and reference ranges for AST across the literature reports was unknown. Methods used for AST measurement in the clinical case reports are almost never given. We do not believe that this potential variation in methods of measurement obviates the utility of the study, as arguably the results are collectively compatible with a simple interpretation of macro-AST distributions as representing two classes of complexes.

In summary, a survey of reported macro-AST concentrations was conducted. The results will enable clinicians to put individual macro-AST results into context of what has been observed, and thus may help prevent unnecessary further testing for some patients. An additional finding was that the reported macro-AST concentrations were reasonably characterized as being of two groups having altered $t_{1/2}$ relative to normal AST.

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