

Original Article

Optimization of the diagnostic capacity of traditional biomarkers in muscle damage and its use in the diagnosis of dermatomyositis and polymyositis

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Keywords

creatine kinase, aldolase, muscle damage, dermatomyositis, polymyositis

Abstract

BACKGROUND-AIM

Creatine kinase (CK) and aldolase are markers traditionally used in the study of muscle damage (MD). As CK determination is more specific to muscle damage, the demand for both determinations in routine laboratory tests would entail an extra cost.

METHODS

Retrospective observational study conducted between 2019-2020. CK and aldolase concentrations from 218 patients were studied.

ROC curves were analyzed for CK and aldolase for muscle damage detection. Cut-off values were selected for both strategies. Specificity of CK and aldolase for dermatomyositis or polymyositis diagnosis in our population was studied using the McNemar's test.

RESULTS

The area under the ROC curve (AUC) for total CK was 0.716 (95%CI: 0.651-0.775), for CK in males it was 0.703 (95%CI: 0.592-0.799), and for CK in females was 0.719 (95%CI: 0.636-0.793). For aldolase, AUC was 0.505 (95%CI: 0.437-0.573). Optimized cut-off points for each determination were: 112 U/L for CK in men, with a sensitivity of 73.9% (95%CI: 51.6-89.8) and a specificity of 49.2% (95%CI: 35.9-62.5); 88 U/L for CK in women, with a sensitivity of 75.0% (95%CI: 57.8-87.9) and specificity of 50.5% (95%CI: 40.4-60.6); and 5.6 U/L for aldolase, with a sensitivity of 61.0% (95%CI: 53.2-68.8) and a specificity of 38.8% (95%CI: 26.5-52.6).

Regarding the individuals diagnosed with dermatomyositis or polymyositis, 66.7% and 44.4% of them were correctly classified as pathological by CK and aldolase results, respectively. McNemar's test did not reveal significant differences.

CONCLUSION

The determination of CK offers a better diagnostic performance of MD and, in addition, does not present significant differences regarding the determination of aldolase in cases of polymyositis and dermatomyositis. Therefore, the single determination of CK would be sufficient for MD screening.

INTRODUCTION

The evaluation of serum muscle enzymes is common in the study of patients presenting muscle weakness or myalgias in whom muscle myopathy is suspected. These enzymes can be elevated in inflammatory myopathies (such as polymyositis and dermatomyositis), infectious myopathies, dystrophinopathies, rhabdomyolysis, and metabolic myopathies, among others [1,2]. Myositis is described as any condition that causes inflammation in skeletal muscles. Its main symptom is muscle weakness, whereas muscular pain is not always present. It can be caused by different entities including infections, autoimmune diseases, and muscle injury [3]. In this context, polymyositis is a rare inflammatory disease that causes muscle weakness, thereby affecting both sides of the body. Having this disease can make it difficult to climb stairs, stand after sitting, lift, or reach for places above your head. Commonly, it affects adults between 30 and 50 years and is more common in African than in Caucasian population. In addition, women are affected more often than men. Signs and symptoms usually appear gradually, over weeks or months. Risk of polymyositis is higher in case of lupus, rheumatoid arthritis, scleroderma, or Sjögren's syndrome [4-6]. While polymyositis has no cure, treatment ranging from medications to physical therapy can improve muscle strength and function [7]. Dermatomyositis, however, as well as being a rare inflammatory disease characterized by muscle weakness, also causes a particular skin rash. It can affect adults and children. In adults it occurs between the age of 45 and 65 years. In children, it usually appears between 5 and 15 years old [4,5,6]. Dermatomyositis also affects women more than men. It has no cure, but there may be periods when symptoms improve. Meanwhile, treatment can eliminate the skin rash and help regain muscle strength and function [7]. Recently, dysregulation in microRNA which normally regulates the immune system has been found in cases of myositis, but further research is needed in order to understand the role of microRNAs in these cases [8].

In clinical laboratories, aldolase and creatine kinase (CK) are two enzymes whose analytical determination has the study of muscle damage as its main usefulness [1]. Aldolase is an enzyme that acts on glucose, allowing energy to be obtained. It is distributed throughout the body, mainly in muscle tissue. The main clinical use of aldolase determination is in the diagnosis and monitoring of musculoskeletal diseases [9,10]. CK is an enzyme located in the inner mitochondrial membrane, myofibrils, and cytoplasm of myocytes. It is involved in the storage and transfer of energy and is the most widely used enzyme for diagnosing and tracking muscle diseases. Its highest serum concentrations are found in response to muscle damage for which it is the most sensitive marker [10,11]. However, regarding polymyositis and dermatomyositis,

an increase in aldolase concentration has been described with no increase in CK concentration [12,13,14]. Myoglobin is a protein that is also released in muscle damage together with CK. However, it has a short half-life and its concentrations start to decrease when CK concentrations are still elevated, thus it is less sensitive if the muscle damage is not recent. Also, myoglobin is most commonly used to study the risk of acute renal injury since high concentrations of this protein can exceed the protein-binding capacity of the plasma causing nephrotoxicity and renal failure. On top of that, determination of this protein is more expensive than other enzymes that appear to be more informative, like CK. That is why it is not a commonly used determination for muscle damage study in clinical laboratories [15,16]. Currently, it is common practice for clinicians to ask for the simultaneous measurement of these two biomarkers in cases of suspected muscle damage. Nevertheless, in our experience, aldolase and CK appear elevated when there is muscle damage. Hence, as CK determination is more specific to muscle damage, the demand for both determinations in routine laboratory tests would entail an extra cost [17,18]. The aim of this study was to optimize the decision limits for CK and aldolase as muscle damage biomarkers in our laboratory, as well as to compare their diagnostic accuracy for this kind of pathologies. Based on the results, a study of the economic cost derived from an incorrect demand for these markers was also carried out.

MATERIAL AND METHODS

A retrospective observational study was performed at Hospital Universitari Son Espases (Palma de Mallorca, Spain), a tertiary level hospital that provides healthcare for an approximate population of 325,000 individuals. The assessed period was between January 2019 to December 2020. Analytical data were obtained from the laboratory information system Gestlab (Indra, Spain), and the clinical information (diagnostic data) was extracted from the hospital information system Millennium (Cerner Corporation), after obtaining approval from the Ethics Board of our institution (Research Ethics Committee of the Balearic Islands (IB 5121/23 PI)). This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. The current decision limits (upper reference limits) in our hospital for serum CK and Aldolase were provided by the manufacturers, Abbott Diagnostics (USA) and BioSystems (Spain), respectively: CK (Male (M) 200 U/L, Female (F) 168 U/mL); Aldolase (7 U/L). Serum CK was quantified by spectrophotometry on the Alinity c platform (Abbott Diagnostics, USA). Long-term imprecision (6 months) was satisfactory, with coefficients of variation (CVs) of 2.5% at 78 U/L, and 3.5% at 660 U/L; whereas aldolase enzyme levels were quantified by spectrophotometry using the BA25 equipment (BioSystems, Spain) with an analytical coefficient of variation of 4.8% (concentration similar to the current decision limit).

Inclusion criteria

Inpatient laboratory requests were considered if they included simultaneous CK and aldolase determinations. Only the first request for each individual with both biomarkers was included in the study. Results were considered pathological when the clinical information reflected an entity of muscle damage as the final diagnosis for that episode.

Diagnostic comparison: CK vs Aldolase

For each individual, age and sex were recorded, and different groups were established according to the diagnosis reflected on the medical records: individuals with non-muscle damage versus individuals with reported muscle damage. In order to determine which diagnostic strategy had a better discriminating power for muscle damage, a receiving operating characteristics (ROC) curve analysis was performed and the area under the curve (AUC) was quantified. Based on the results, cut-off values (for CK (by sex) and aldolase) were selected for the maximization of the diagnostic performance. Cut-off values with maximal discrimination power were used to calculate Cohen's Kappa index and the odds ratio (OR) for muscle damage. Once cut-off points were optimized, we checked whether aldolase was the more specific enzyme for dermatomyositis or polymyositis diagnosis in our population, as described in the literature. In order to verify this fact, we evaluated the proportion of well-classified (dermatomyositis/polymyositis versus non-muscle damage) individuals according to CK and aldolase concentrations.

Economic implications

Using only one enzyme, either CK or aldolase when muscle damage is suspected, would lead to economic savings. Evaluation of such savings was also performed retrospectively, considering a unit price of 2 euro for each determination (CK and aldolase).

Statistical Analysis

AUCs were compared using the Bamber methodology. Comparison of proportions for related samples was performed using the McNemar test. SPSS v.24 (IBM Corporation, USA) and MedCalc v.19.3 (Belgium) software were used for all calculations. Statistical significance was set at 0.05.

RESULTS

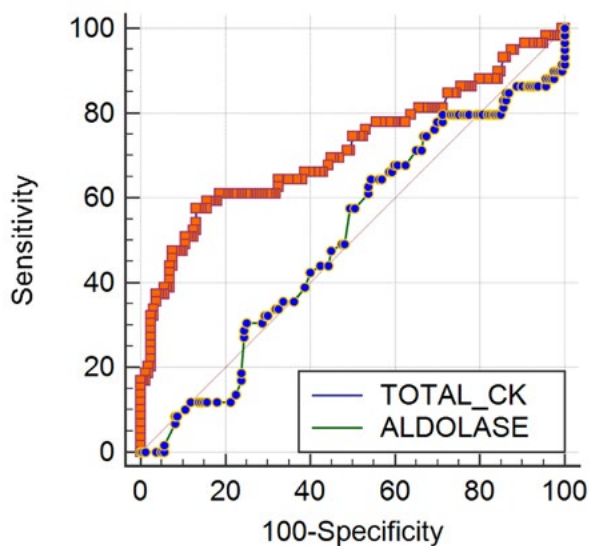
During the period studied, in a total of 1502 individuals, CK and aldolase determinations for muscle damage were simultaneously requested, 218 of whom were inpatients. Sixty-eight of them (31%) were considered pathological in view of the final diagnosis reflected in their medical records. The diagnosis included mainly: myopathy (15%), myelitis (9%), dermatomyositis and polymyositis (26%), Behçet syndrome (7%), myalgia (14%), rheumatoid arthritis (12%), physical exercise (1.5%), Kawasaki syndrome (1.5%), ELA (1.5%), chronic inflammatory demyelinating polyneuropathy (1.5%), seizures (1.5%), and Duchenne syndrome (1.5%). Anthropometric and biochemical features are described in Table 1. The area under the ROC curve (AUC) for total CK was 0.716 (95% CI: 0.651-0.775). However, by sex, for CK in males it was 0.703 (95% CI: 0.592-0.799), whereas AUC for CK in females was 0.719 (95% CI: 0.636-0.793). For aldolase, AUC was 0.505 (95% CI: 0.437-0.573). Comparison of ROC curves of total CK and aldolase is shown in Figure 1. In addition, statistically significant differences were found between CK (male and female) and aldolase AUCs (p -value<0.01). Optimized cut-off points for each determination were: 112 U/L for CK in men, with a sensitivity of 73.9% (95% CI: 51.6-89.8) and a specificity of 49.2% (95% CI: 35.9-62.5); 88 U/L for CK in women, with a sensitivity of 75% (95% CI: 57.8-87.9) and specificity of 50.5% (95% CI: 40.4-60.6); and 5.6 U/L for aldolase, with a sensitivity of 61% (95% CI: 53.2-68.8) and a specificity of 38.8% (95% CI: 26.5-52.6). Upon applying the optimized cut-off points of CK and aldolase, Cohen's Kappa index for women was 0.51, while for men it was 0.19, for any diagnosis of muscle damage. Similarly, the following odds ratios were obtained for general muscle damage: CK (M) 2.7 (95% CI: 1.0 – 7.8); CK (F) 3.1 (95% CI: 1.4 – 7.1); aldolase 1.0 (95% CI: 0.5 – 1.8). After studying individuals diagnosed with dermatomyositis or polymyositis (26% pathological diagnosis), and in consideration of the CK results, 66.7% (12/18) presented a value higher than the established cut-off value and, therefore, were correctly classified as pathological. However, regarding aldolase results, only 44.4% (8/18) were correctly classified as pathological. Despite these differences, McNemar's test did not reveal significant differences between both biomarkers (p -value = 0.344). Over the two years of the study, a total of 1502 aldolase determinations were co-processed with CK, amounting to a total cost of 3004 euro for this extra determination.

Table 1: Descriptive statistics of the studied population

Diagnosis	Sex (Male/Female)	Age: Median (years, IQR*)	Median CK (U/L, IQR)	Median Aldolase (U/L, IQR)
Pathological (31%)	22/46	42 (13-53)	122 (82-318)	6.3 (4.6-8)
Non Pathological (69%)	58/92	11 (6-21)	94 (67-142)	6.1 (4.6-8.7)

*IQR: interquartile range

Figure 1: ROC curves (total CK and aldolase) for diagnosis of muscle damage



DISCUSSION

CK and aldolase are two analytical determinations that are used for the diagnostic study of muscle damage, in which an increase in these biomarkers can occur. It is described that the only cases in which an elevation of the enzyme aldolase could occur without elevation of the enzyme CK are those of dermatomyositis and polymyositis [12-14]. Currently, it is common to simultaneously determine both biomarkers to study muscle damage, which could lead to unnecessary health expenditure if both determinations offer the same information. According to the literature, this happens only in the specific cases of dermatomyositis and polymyositis, the incidence of which is not very high – presenting 4.7-5.2/106/year for dermatomyositis and 3.7-4.1/106/year for polymyositis in Spain, in general [19]. To study the diagnostic capacity of both biomarkers, 218 patients were studied, from whom the ROC curves of CK, separated by sex, and aldolase were analyzed. Analysis of the ROC curve of both biomarkers indicated that the determination of aldolase for muscle damage offered no

information and, further, the diagnostic performance for muscle damage of CK was significantly higher than the diagnostic performance of aldolase. This fact is also highlighted through the odds ratio obtained for both biomarkers. Furthermore, according to international recommendations [20], each laboratory should establish its own reference values and decision limits. In this way, new cut-off values of both determinations were obtained. These decision limits were used to study the concordance of both biomarkers for muscle damage. However, the fact that the cut-off point obtained for CK is around the median of the normal value, highlights both, that the reference values provided by the manufacturer are not well characterized or do not suit to our population, so it would be necessary to further study and properly characterize our population with good reference ranges. And that, even if CK seems to be a better biomarker for the study of muscle damage than aldolase, it is far from being an ideal biomarker due to its lack in distinguishing between pathological and non-pathological patients.

Finally, since in the cases of dermatomyositis and polymyositis an elevation of aldolase has been described without the presence of CK elevation, the differences between both markers were evaluated for 18 cases of dermatomyositis and polymyositis. Positive results were observed in 12 of the 18 cases for CK, representing 66.7% of cases, and in 8 for aldolase, representing 44.4%. No significant differences were found, in our population, between the measurement of CK and aldolase for the described cases of polymyositis and dermatomyositis. These results show that, in our population, the use of aldolase for detection of cases of dermatomyositis and polymyositis does not offer more information than the use of CK. However, it would be necessary to continue the study by increasing the number of cases to confirm these results. Globally, this study has some limitations, mainly related to its retrospective nature and the confidence in the records obtained from the LIS and hospital information system. Besides, the number of cases studied was low, especially in the cases of dermatomyositis and polymyositis. From an economic point of view, the single request of CK determination in case of diagnostic suspicion of muscle damage would be enough and would lead to financial savings. It is important to highlight that the study was carried out during a period corresponding to the COVID-19 pandemic, with a greater expense being likely in non-pandemic operating conditions of the laboratory.

CONCLUSION

The results obtained in this study indicate that the determination of CK offers a better diagnostic performance of muscle damage and, in addition, does not present significant differences regarding the determination of aldolase in cases of polymyositis and dermatomyositis, in which an increase in aldolase without an increase in CK has been described. Therefore, the single determination of CK would be sufficient for muscle damage screening and would mean a decrease in health expenditure. Based on the 'right care' philosophy, clinical laboratories need to offer not only true results, but also become a cornerstone in the optimization of resources.

Research Funding

This study has not received any type of public or private funding.

Author contributions

Sara Sanchez Asis: Conceptualization, Investigation, Methodology, Software, Supervision, Validation, Visualization, Data curation, Formal analysis, Writing original draft - review & editing. **María Cristina Gomez Cobo:** Formal analysis. **David Ramos Chavarino:** Formal analysis. **Beatriz Garcia Garcia:** Formal analysis. **Isabel Llompart Alabern:** Supervision, Validation. **Jose Antonio Delgado Rodriguez:** Investigation, Methodology, Supervision, Validation, Visualization, Writing - review & editing.

Conflict of interest

All authors declare no conflicts of interest.

Ethics approval and consent to participate

The study was approved by the Ethics Board of our institution [Research Ethics Committee of the Balearic Islands (IB 5121/23 PI)]. This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Consent for publication

Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

Data availability

This is a study performed at Hospital Universitario Son Espases (Palma de Mallorca, Spain). Analytical data were obtained from the laboratory information system GestLab (Indra, Spain), and the clinical information was extracted from the hospital information system Millennium (Cerner Corporation, USA).

Acknowledgements

Not applicable

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