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Get reliable laboratory findings – how to recognize the deceptive effects of angiotensin-converting enzyme inhibitor therapy in the laboratory diagnostics of sarcoidosis?

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Abstract

Objectives: Serum angiotensin-converting enzyme (ACE) is the only biomarker routinely used in the laboratory diagnostics of sarcoidosis, and ACE inhibitor (ACEi) drugs are among the most prescribed drugs worldwide. Taking ACEi can mislead medical teams by lowering ACE activity, delaying diagnosis and giving a false impression of disease activity of sarcoidosis. We aimed to develop a simple method to detect the presence of ACEi drugs in samples, to investigate the ACEi medication-caused interference and consequences in a retrospective study.

Methods: ACE activity and the level of ACE inhibition were determined for 1823 patients with suspected sarcoidosis. These values were compared with the therapeutic information at the first and follow-up visits.

Results: A total of 302 patients had biochemical evidence of an ACEi drug effect during diagnostic ACE activity testing. In their case, ACE activity was significantly lower (median(IQR): 4.41 U/L(2.93–6.72)) than in patients not taking ACEi (11.32 U/L(8.79–13.92), p<0.01). In 62 sarcoidosis patients, the ACEi reduced ACE activity to the reference range or below. Only in 40 % of the cases was the medication list recorded in the outpatient chart and only in 3 cases was low ACE activity associated with ACEi use. 67 % of the repeated ACE activity measurements were also performed during ACEi therapy.

Conclusions: Our study revealed that the use of ACEi is common in patients with suspected sarcoidosis. The ACE activity lowering effect of ACEi drugs may escape the attention of medical teams which can lead to diagnostic errors and unnecessary tests. Nevertheless, these pitfalls can be avoided by using a method suggested by our team.

Keywords: angiotensin converting enzyme (ACE); sarcoidosis; ACE inhibitor (ACEi)

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Introduction

Angiotensin-converting enzyme (ACE) inhibitors (ACEi) have been among the five most commonly prescribed drugs in the United States and the European Union for many years [1–3]. This is not surprising, as ACEi are successfully used in the long-term treatment of cardiovascular diseases (e.g. hypertension, heart failure, stroke) [4, 5] or in the prevention of renal complications in diabetes mellitus [6, 7]. ACE not only plays an important role in the pathogenesis of these diseases [8–10] with high morbidity and mortality [11, 12], but also serves as a serum biomarker for diseases associated with granuloma formation [13, 14], in particular for sarcoidosis [15, 16].

Despite intensive research, sarcoidosis is still a disease of unknown aetiology [17], mostly affecting the middle-aged

population [18–21]. To objectively establish the diagnosis of sarcoidosis, histological sampling, which intervention is a burden for the patient [22], and the detection of non-caseating granulomas can be essential, especially in sarcoidosis cases with non-typical clinical presentation and if the disease affects only the lung parenchyma [23, 24]. Detection of elevated serum ACE activity plays a pivotal role in supporting the diagnosis of sarcoidosis [16, 25], moreover decreased ACE level also serves as a cost-effective biomarker in the assessment of response to therapy[26, 27] and monitoring disease activity [28]. Nevertheless, ACEi drugs can also reduce serum ACE activity, thereby limiting the value of ACE activity as a biomarker in sarcoidosis [29–31]. In addition, there has been a noticeable shift toward diagnosing sarcoidosis in the older population in recent decades [21], where sarcoidosis independent ACEi therapy is more likely [32]. ACEi drugs potentially complicate the evaluation of ACE activity findings, prompting extra attention from medical teams, since the false low serum ACE activity values – due to ACEi therapy – can lead to misinterpretation of laboratory findings in sarcoidosis patients.

Here, we aimed to present a simple biochemical method that can objectively identify the effect of any type of ACEi drug in the sample, thus helping clinicians to properly evaluate the laboratory results (without checking the drug prescriptions). Indeed, in our retrospective clinical analysis we show that diagnostic blood samples often contain ACEi drugs and clinicians are typically unaware of the concurrent bias.

Materials and methods

Subjects, clinical data and ethical approval

The retrospective study included patients who underwent a sarcoidosis investigation and diagnostic serum ACE activity determination (n=1853) at the University of Debrecen, Clinical Centre (Debrecen, Hungary), between June 01, 2014 and December 31, 2021. Thirty patients were excluded due to unsatisfactory patient documentation or missing measurement reports. Patients gave informed consent to medical examination, blood sampling and ACE activity determination during their outpatient care. Only data recorded and stored in the medical (UDMed, ver. 5.46, University of Debrecen, Hungary) and in the laboratory informatics systems (GLIMS, ver. 8.11, CliniSys, Belgium) were processed retrospectively. To protect patients' personal data, unique 8-character barcodes were used to ensure patients' anonymity from the evaluators. The retrospective clinical study and the processing of clinical data were approved by the Regional and Institutional Ethics Committee, Clinical Centre, University of Debrecen. Research authorization number: 5925-2021. The research was in accordance with the tenets of the Helsinki Declaration.

Serum samples, ACE activity measurement and level of ACE inhibition

Blood samples were drawn at the time of outpatient presentation using a standard aseptic technique. Separation of serum samples was performed at the Department of Laboratory Medicine, University of Debrecen, Hungary after centrifugation (15 min; 2000 *g*; +4 °C) following coagulation of native blood samples. Serum samples were stored at −20 °C until determination of ACE activity and ACE inhibition. The stability of sample ACE activity between storage conditions was tested for 123 samples. After an average of 6 years and 4 months (min: 5 years 1 month, max: 7 years 3 months), no decrease in ACE activity was observed (baseline ACE activity: 9.0 ± 4.0 U/L, current ACE activity: 9.8 ± 4.2 U/L).

ACE activity was determined using the optimized fluorescent kinetic method previously reported by our group [33]. In addition to the 35-fold serum dilution for diagnostic purposes, ACE activity was measured at 4-fold and 400-fold serum dilutions to determine the degree of ACE inhibition by the following equation:

$$\text{ACE inhibition}\ (\%) = 100 - \left(\text{ACE activity}_{\text{4-fold dilution}} \Big/ \text{ACE activity}_{\text{400-fold dilution}}\right)$$

The presence of an ACEi drug in the serum sample is indicated by a level of ACE inhibition exceeding 80 % [34].

The value of serum ACE activity is significantly affected by the presence of any ACEi drugs in the sample, and the degree of sample dilution applied during the measurement (Figure 1). Human serum albumin reversibly inhibits ACE, which can be eliminated by diluting the sample at least 35-fold during measurement [33]. Below 35-fold sample dilutions, ACE activity is underestimated due to albumin-mediated inhibition, but above 35-fold dilution, the dilution corrected ACE activity becomes constant and reliably measurable (circles, Figure 1). A further increase in ACE activity can be observed above 35-fold sample dilution in sera containing an ACEi drug (squares, Figure 1). ACE activity values measured at 35-fold dilution often do not distinguish between patients taking ACEi and those not taking ACEi (symbols highlighted in green, Figure 1), especially if measured values are within the normal reference range. From the ACE activity values measured at 4-fold and 400-fold serum dilutions (inhibited and almost uninhibited activities, respectively), the degree of ACE inhibition can be accurately expressed. This provides information on the albumin-mediated endogenous ACE inhibition in patients not taking ACEi (level of inhibition=55 %, Figure 1) and on the effect of ACEi drugs in patients taking an ACEi medication (level of inhibition=97 %, Figure 1). Based on the level of maximal endogenous ACE inhibition (~78 %, [34]), in case the measured ACE inhibition is 80 % or more it indicates the presence of an ACEi drug.

Statistical analysis

Normal distribution was assessed using D'Agostino-Pearson omnibus normality test. All values with normal distributions are shown as mean (± standard deviation), while those with non-normal distributions are expressed as median (range), for which range is the 25–75th percentile. Normally distributed data were compared using unpaired t-test with Welch's correction, while data with non-normal distribution were compared using Mann–Whitney U test. Statistical analysis was performed using GraphPad Prism software, version 9 (San Diego, CA, USA). p-values <0.05 were considered statistically significant.

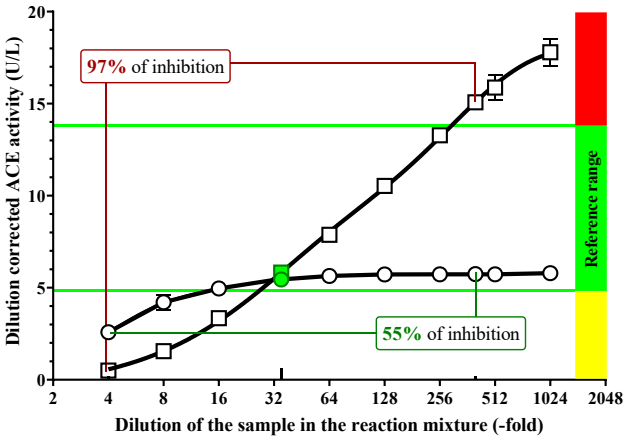

Figure 1: The effect of an ACE inhibitor drug can be reversed by increasing the dilution of the serum sample. Dilution corrected changes in serum ACE activity in a patient taking ACE inhibitor (square) and a patient not taking ACE inhibitor drug (circle) measured at different serum dilutions. The symbols for ACE activity values (measured at 35-fold dilution) presented in the laboratory report are highlighted in green. Each symbol denotes the mean and standard deviation of three independent determinations. The reference range of serum ACE activity (measured at 35-fold sample dilution) and the level of ACE inhibition in the two samples are indicated.

Results

We wanted to confirm that the 1823 patients with suspected sarcoidosis were not under the effect of any ACEi drugs at the time of blood sampling. We assumed that the clinicians would be cautious during sampling (changing the patients' ACEi medication to an angiotensin receptor blocker or temporarily stopping therapy before sampling), as the aim of the test is to support the suspicion of sarcoidosis by elevated serum ACE activity. Surprisingly, one in 6 patients (302 patients out of 1823) included in the study had biochemical evidence of an ACEi drug effect during diagnostic ACE activity testing (red columns, Figure 2A). The ACEi drug significantly increased the level of ACE inhibition (94.9 % (91.6–96.7 %)) compared to endogenous, albumin-mediated ACE inhibition (52.9 % (46.9–59.85 %)). As a result, serum ACE activity was significantly lower in the ACEi treated group (4.41 U/L (2.93–6.72 U/L)) than in patients not taking an ACEi (11.32 U/L (8.79–13.92 U/L), $p<0.01$), measured at 35-fold serum dilution (Figure 2B). We could not infer the presence of an ACEi drug just from a single ACE activity value, as ACE activity values of 77.5 % of patients (n=234) treated with

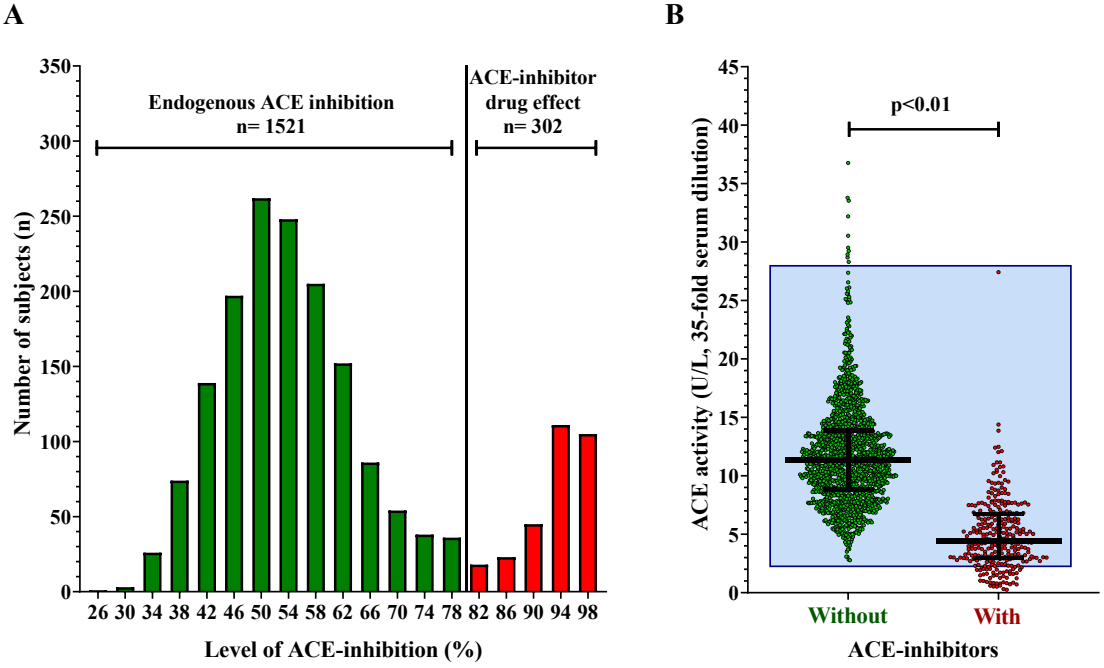

Figure 2: The distribution histogram of ACE inhibition showed that every 6th patient was under ACE inhibitor treatment at the time of sampling (A), resulting in low ACE activity (B). The green bars show the distribution of patients with normal endogenous ACE inhibition, while the red bars show the distribution of patients with increased ACE inhibition due to ACE inhibitor treatment (A). Each dot represents a patient's ACE activity value (B), the blue area highlights the overlapping activity range. The median and interquartile range of ACE activity in patients taking and not taking ACE inhibitors are also shown.

an ACEi drug overlapped with patients not taking an ACEi (blue rectangle, Figure 2B).

The degree of serum dilution in the reaction mixture impacts the measured ACE activity significantly, which could be observed also in our study population (Figure 3). Compared with 4-fold serum dilution, the dilution-adjusted ACE activity measured at 35-fold dilution is significantly higher (+99 %; 5.68 U/L (4.31–7.29 U/L) vs. 11.32 U/L (8.79–13.92 U/L)) due to the elimination of the reversible endogenous inhibitory effect of albumin (Figure 3A). In comparison, the effect of further sample dilution on ACE activity is negligible (+8 %, 12.23 U/L (9.20–15.60 U/L) at 400-fold dilution). In contrast, ACE activity measured at 400-fold dilution of ACEi-containing samples resulted in an even more significant increase in ACE activity (+125 %). The activity measured at 400-fold dilution approached the ACE activity that the patient would have without ACEi medication (Figure 3B).

ACE activity determination helps to diagnose sarcoidosis when it is above the reference range for the normal population. ACE activity values measured at 400-fold dilution were used to determine the number of patients where ACEi drug masked high ACE activity. According to our results, 2 patients (out of 161 patients whose ACE activity was initially below the reference range, measured at 35-fold dilution) would have had ACE activity above the reference range at 35-fold dilution (Figure 4A). However, among the 138 patients whose ACE activity remained within the normal range at this dilution level, 60 would have had ACE activity exceeding the reference range without ACEi treatment (Figure 4B). Based on these results, measurement of ACE activity could have supported the diagnosis of sarcoidosis in at least 62 more cases if the patients had not been on ACEi therapy at the time of sampling.

A careful review of the 302 patients' medical records showed that patients treated with ACEi are older than patients not treated with ACEi, both between women (60.5 ± 11.7 years vs. 48.2 ± 14.0 years; $p<0.01$) and men (55.4 ± 13.3 years vs. 44.3 ± 15.1; $p<0.01$, Table 1). Furthermore, there were only 121 cases where the patients' medication was recorded, which would have allowed the medical team to identify the use of an ACEi drug. Although the fact of a lower-than-expected ACE activity was documented in 50 cases, the underlying ACEi medication was only recognized in 3 cases (Table 1).

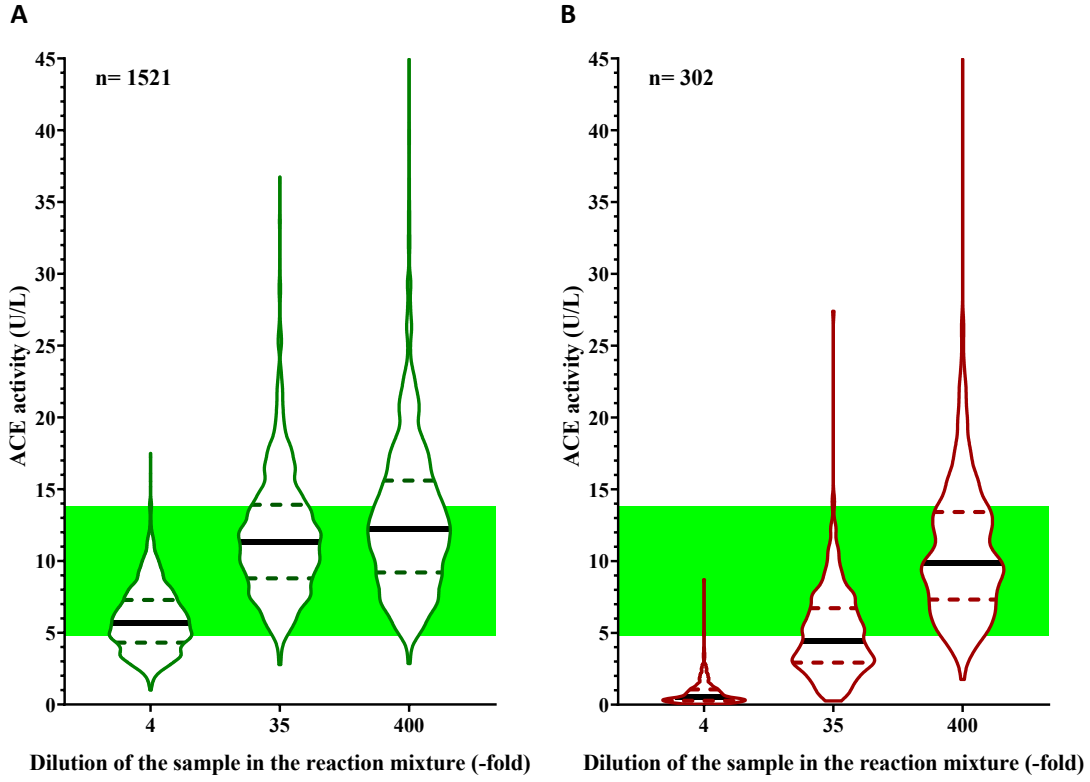

Figure 3: Violin plots illustrating the distribution of ACE activities measured at relevant dilution values (4, 35, 400× dilution) in patients not taking ACE inhibitors (A) and patients taking ACE inhibitors (B). The horizontal solid lines indicate the median values for the groups, the thick green lines indicate the interquartile ranges. The green area indicates the normal range of ACE activity measured at 35-fold dilution.

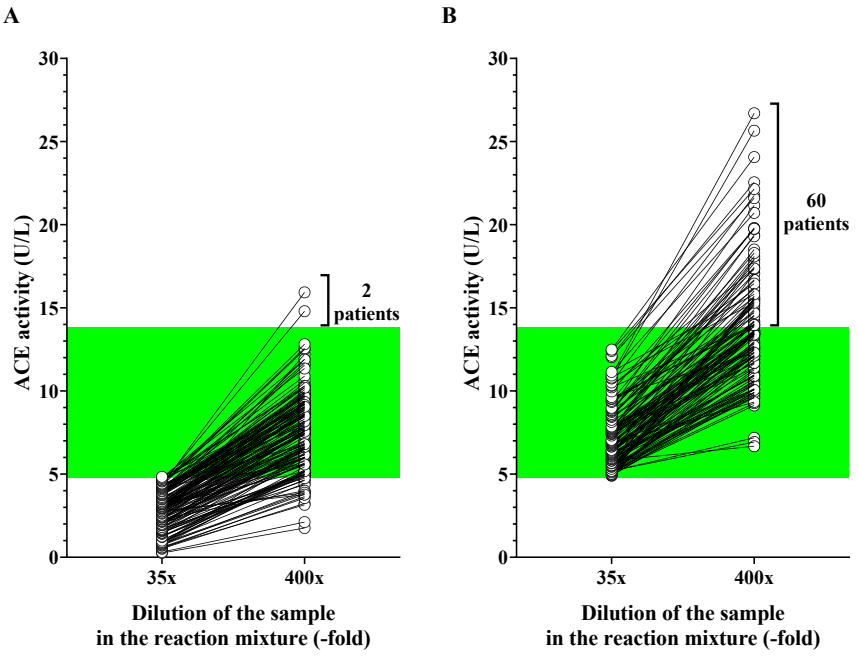

Figure 4: In at least 62 patients, the serum ACE activity would have been above the reference range if the patient had not been on ACE inhibitor therapy. Each pair of symbols show the ACE activity values for a single patient measured at 35 and 400-fold dilutions for patients with ACE activity below the reference range (at 35-fold dilution, A) and for patients within the reference range (at 35-fold dilution, B).

Table 1: The behavior of medical teams in the light of ACEi treatment. For each question, the number of cases concerned is indicated (n). Values with normal distributions are shown as mean (± standard deviation), while those with non-normal distributions are expressed as median (IQR).

	Population	Women		Men	
		ACE-inhibitor treatment		ACE-inhibitor treatment	
		Yes	No	Yes	No
n	1823	171	811	131	710
Age, years	46.6 (38.8-58.9)	60.0 (53.4-70.3)	46.8 (39.7-57.9)	55.4 ± 13.3	42.0 (35.9-52.1)
ACE activity, U/L	10.39 (7.5-13.4)	4.36 (2.9-6.7)	10.89 (8.4-13.4)	4.56 (2.9-6.8)	11.77 (9.4-14.6)
ACE-inhibition, %	55.1 (47.1-66.7)	95.0 (91.8-96.7)	51.6 ± 10.6	94.8 (90.6-96.7)	53.9 ± 10.9

Was the medication indicated on the outpatient form?	Yes:	121	No:	181
Type of ACE-inhibitor:	perindopril:	92 (76%)		
	ramipril:	16 (13%)		
	enalapril:	10 (8%)		
	lisinopril:	3 (3%)		
Was the result interpreted on the clinical report?	Yes:	50	No:	252
Had the ACE inhibitor effect been recognised?	Yes:	3	No:	299
Had a control ACE activity measurement been performed?	Yes:	43	No:	259
Was there a detectable ACE inhibitory effect?	Yes:	29	No:	14

We identified patients who were first sampled during ACEi treatment and whose physician requested a repeat sampling for ACE activity determination (Table 1). In 29 out of 43 patients, repeated ACE activity measurements were also performed under ACEi treatment, ACE inhibition levels remained above 80 % (94.1 ± 3.8 % vs. first presentation: 93.4 ± 3.9 %, p>0.05) and ACE activity did not change from baseline during follow-up visits (4.62 ± 2.1 U/L, 5.16 ± 2.5 U/L,

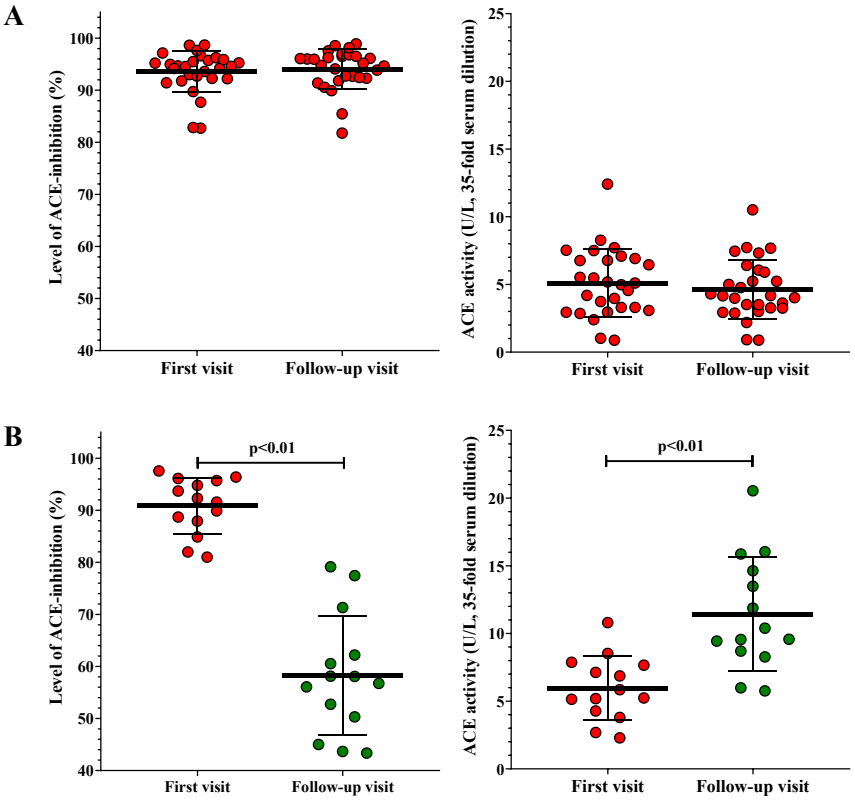

Figure 5: In some of the patients, ACE activity measurements were repeated during continued ACE inhibitor treatment. In patients where ACE inhibitor treatment was not discontinued and ACE activity measurements were repeated, high ACE inhibition (left graph, A) and low ACE activity (right graph, A) were still measured. Discontinuation of ACE inhibitors led to a significant decrease in level of ACE inhibition (left graph, B) and an increase in ACE activity (right graph, B). Symbols show the ACE activity value for a single patient, and the mean and standard deviation of the groups are also indicated.

p>0.05, respectively; Figure 5A). Conversely, no biochemical evidence of an ACE inhibitory effect was found at follow-ups in 14 cases (ACE inhibition level: 90.1 ± 5.4 % vs. 58.2 ± 11.5 % at second presentation, p<0.01), accompanied by a marked increase in serum ACE activity (6.0 ± 2.4 U/L vs. 11.4 ± 4.2 U/L at second presentation, p<0.01, Figure 5B).

Discussion

Diagnosing sarcoidosis is often a challenge for medical teams and is not helped by the fact that ACE is the only biomarker mentioned in the WASOG international guidelines for the diagnosis and follow-up of patients with sarcoidosis [35]. In addition, reducing ACE activity is a key target in the treatment of patients with cardiovascular diseases, making ACEi one of the most prescribed and used drugs worldwide [1–3]. Falsely low ACE activity in sarcoidosis caused by ACEi may confuse clinicians, delay diagnosis and induce unnecessary investigations. To prevent this, it is crucial to have a knowledge of ACEi usage and a thorough evaluation of ACE activity values. To date, patients' report remain the primary source of ACEi usage.

A simple and easy way to measure the effect of reversible enzyme inhibitors (like ACEi) in biological samples is to determine the enzyme activity at different sample dilutions during analysis. With increasing dilution of the sample, the concentration of the reversible inhibitor decreases in the reaction mixture, leading to a gradual loss of its inhibitory effect. Optimally, it is possible to obtain a condition by significantly diluting the sample, as if the patient were not taking the drug, so that the level of uninhibited enzyme activity in the patient can be objectively determined without interrupting the medicinal treatment.

We used this method to investigate whether or not ACE activity measurements with suspected sarcoidosis were executed during ACEi treatment at an academic medical centre for over 1800 patients. Surprisingly, our measurements confirmed that one in six patients were on ACEi treatment at the time of sampling and therefore had artificially (drug mediated) low ACE activity as determined by the conventional laboratory ACE test. ACE activity below or not above the reference range could alert clinicians to inappropriate sampling conditions in this population. Unfortunately, it seemed that medical teams did not pay enough attention to the effect of ACEi drugs in reducing ACE activity, because we also identified patients in our study who were continuously ordered to have ACE activity measurements albeit ACEi treatment was not interrupted. Of course, a possible explanation might be that some of the patients could not name all the drugs they were taking, so the clinicians may not have been aware that the drugs the patient

was taking were affecting the laboratory results. These demonstrate the need for an objective test, which can be used by the laboratory expert for the correct interpretation of the measured values from a single blood sample, without the need for any clinical data on medication. Moreover, the laboratory can provide feedback to the medical team with the objective data, highlighting ACEi drug interference and ways to avoid it, reducing unnecessary tests, caregiver workload and patient frustration.

In cases supported by measurement results, the ACE activity value of a patient taking an ACE inhibitor is recommended to be provided as an interpretative report for information purposes only. In it, laboratory professional should draw the attention of the medical team to the fact that the sample is likely to contain an ACE inhibitor drug that significantly reduces ACE activity. However, we should leave it to the medical team to decide how to modify the drug therapy before the next ACE activity measurement, as the withdrawal of ACE inhibiting drugs, even for a short period, may increase the patient's cardiovascular risk (e.g. due to a dramatic increase in blood pressure). Nevertheless, it should be emphasised that other types of drugs that act on the renin-angiotensin-aldosterone system (e.g. angiotensin receptor blockers) have no effect on serum ACE activity and therefore do not interfere with ACE activity measurements.

Our results confirm – not for the first time in the literature – that mean ACE activity is lower in patients taking ACEi than in those not taking ACEi when examined at the population level [36–40]. However, in contrast to previously published papers, we would also like to emphasize that at the individual level there is a significant overlap between individual ACE activity values in patients taking or not taking ACEi drugs (77.5 % of patients studied had serum ACE activity in the same range regardless of use of ACEi medications). As a result, it has been nearly impossible to elucidate which of the samples with ACE activity within the normal range originated from a patient with otherwise high ACE activity who was treated with an ACE inhibitor. Detection of ACEi drugs from serum is mostly done using expensive and instrument-intensive methods such as mass spectrometry. Additionally, detection of dozens of ACEi drug molecules on the market requires unique methods with their own calibrators and controls, making routine use of these measurements unfeasible. However, the method presented here can be used for any type of ACEi drug taken at any time of day, as it measures the effect of the drug, rather than identifying the molecule itself (Supplementary Figure 1) [41, 42]. In fact, since the determination is still based on ACE activity measurements, it can be easily applied in any laboratory where ACE activity measurements can be performed (Supplementary Figure 2) [43] without the need to purchase any new equipment or to set up new methods.

In this study, 80 % was taken as the cut-off level for ACE inhibition, above which ACE inhibitor treatment is likely. The determination of the cut-off value was guided by the level of endogenous ACE inhibition in the local population, which in our case did not exceed 80 % in any individual (maximum 78 %). However, we would like to point out that the level of endogenous ACE inhibition may differ among populations, so it is worth verifying the level of endogenous ACE inhibition in the local population in an ACE inhibitor free population before using this cut-off value.

This is not the first publication to reveal the problem of ACEi in sarcoidosis diagnostics, but to our knowledge it is the first to suggest a routinely applicable method for identifying patients taking ACEi. A study in Belgium found a rate of around 10 % [44], while a retrospective study in Iowa found a rate of around 8 % of patients who had ACE activity testing while on ACEi therapy [30]. It is worth pointing out, however, that these studies could only be based on data recorded in medical documentation and extremely low ACE activity values (not all patients were tested for drug levels). We found this rate to be higher, around 17 %, which is probably due to the biochemical analysis of the whole study population and shows the true magnitude of the problem.

The method presented here is an effective way to objectively estimate the effect of ACE inhibitor drugs. However, it is worth noting that the quality of the serum sample is not negligible in the ACE activity measurements. For example, hemolysis of the sample results in an underestimation of basal ACE activity [33] and artificially increases the measured inhibition. All serum samples were analysed qualitatively (using serum indices [33]), resulting in the rejection of ACE activity ordering in the presence of significant interference, and these samples were not included in our study. Furthermore, in the case of ACE inhibitors containing a free sulfhydryl group (e.g. captopril), dimerization of the drug and oxidation of the sulfhydryl group can be expected during prolonged storage (weeks, months) which results in loss of ACE inhibitory ability [45, 46]. For this reason, samples containing ACE inhibitor drugs with a sulfhydryl group can be expected to show a slow *in vitro* decrease in the level of ACE inhibition.

Interpretation

ACE activity measurement for diagnostic purposes is often performed in patients who are on ACEi medication. An ACEi drug can mislead the medical team monitoring sarcoidosis

by ACE activity, providing false information about the activity and severity of the disease. Using the method presented here, the effect of any ACEi can be objectively detected without data on medication. Additionally, this method can be easily set up in laboratories where ACE activity is measured since it does not require any new equipment or reagents, thus eliminating potential diagnostic errors caused by ACEi drugs in the diagnosis and follow-up of sarcoidosis.

Research ethics: The retrospective clinical study and the processing of clinical data were approved by the Regional and Institutional Ethics Committee, Clinical Centre, University of Debrecen. Research authorization number: 5925-2021. The research was in accordance with the tenets of the Helsinki Declaration.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards. Patients gave informed consent to medical examination, blood sampling and ACE activity determination during their outpatient care.

Author contributions: Sample handling: TBP, ISM, PH. Biochemical measurements: ISM, AÁSz, EEE, ITA, Conceptualization and study design: MF, AÁSz. Data handling, analysis and statistics: AÁSz, MF. Interpretation: MF, AT, ZP. First manuscript draft: MF, AÁSz, EEE. Review of manuscript: EB, CsV, MF, AT, PZ.

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Data availability: The raw data can be obtained on request from the corresponding author.

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