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Comparative analysis of high CRP-levels in human blood using point-of-care and laboratory-based methods

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ABSTRACT

Objectives: The use of point-of-care (POC) methods and the measurements of C-reactive protein (CRP) as a diagnostic marker have both increased over the past years. This has led to an increase in POC-methods analysing CRP. High CRP levels are often seen as an indication for the subscription of antibiotics. The quality of POC-systems compared to routine diagnostic measurements for the analysis of CRP is thereby of main importance, since many small practises will use POC-methods. This study compared high-level CRP concentrations (above 100 mg/L) using an i-CHROMA^{TM} with 2 routinely used laboratory-based systems (Architect and ABX).

Design: and Methods: A total of 199 patient samples with a CRP concentration above 100 mg/L were analysed with the i-CHROMA^{TM} POC system and the turbidimetric routine methods using the Architect and ABX equipment.

Results: The results of the i-CHROMA^{TM} device showed a significant decrease in the CRP levels compared to those obtained with the Architect and the ABX (i-CHROMA^{TM} vs. Architect: $y = 0.6792x + 94.701$; $R^2 = 0.4980$, i-CHROMA^{TM} vs. ABX: $y = 0.3674x + 118.05$; $R^2 = 0.3964$, Architect vs. ABX: $y = 0.7657x + 36.337$; $R^2 = 0.9311$). Furthermore, data analysis showed a partition of the i-CHROMA^{TM} measurements in two defined clouds, which could not be explained with any of the available sample information.

Conclusions: This analysis showed the limitations of the i-CHROMA^{TM} CRP analyser. In addition, it illustrates the need for strict regulations on the information and output provided by companies regarding the boundaries of novel and existing diagnostic methods.

1. Introduction

Due to the rapid increase of the C-reactive protein (CRP) concentration in the event of an inflammatory disease, CRP is regarded as one of the most important acute phase proteins [1]. Inflammation and CRP play an important role in operations, trauma, mental stress, myocardial infarction and neoplastic diseases [2]. CRP also acts as a pattern recognition receptor for the initiation of the activation of humoral and cellular effector pathways. Furthermore, it plays an important physiological role in the host defence and in the activation of the complement system [3].

Compared to traditional markers, such as sedimentation rate and leukocyte count, CRP, with a half-life of 19 hours, is more sensitive

Abbreviations: (POCT), Point-of-care testing; (CRP), C-reactive protein.

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for the detection of inflammatory processes. In healthy individuals, the concentration of CRP should be below 5 mg/L. Higher CRP values normally indicate the presence of a bacterial infection or other inflammatory events [4].

In order to ensure a rapid diagnosis and thus avoid unnecessary antibiotic treatment, there are many different methods on the market for the detection of CRP levels in human blood. The most common detection methods are turbidimetric assays, lateral flow assays, sandwich immunoassays, fluorescence assays, chemiluminescence assays, electrochemical assays and novel lab-on-a-chip based immunoassays [3]. In clinical diagnostic laboratories, the detection of CRP is performed using high throughput analysers such as the Architect ci8200SR (Abbott, Chicago, USA). In contrast, analysers such as the ABX Micros CRP 200 from Axonlab (Baden, Switzerland) are often used by general practitioners (low throughput) or as a back-up analyser to measure CRP in clinical laboratories. Since these methods are relatively time intensive and require trained personnel to perform, CRP is also often detected by using point-of-care testing (POCT) methods, which are easy to use and reduce the turnaround time significantly. Surgery units, ambulances, paediatric practices and primary care physicians already work with a range of different POCT methods [5].

As for many other parameters, there are already several different POCT devices for the measurement of CRP. One example of such a device is the i-CHROMA^{TM} (Boditech Med Inc., South Korea). Previous studies have shown that the performance of the available CRP-POC systems vary [6–10]. The data obtained with the i-CHROMA^{TM} CRP-POC device was by most studies shown to be comparable to other detection methods at lower concentrations, whereas a trend was found towards non-comparable measurements at higher concentrations (>100 mg/L). However, the statistics for the measurements at higher CRP levels were hindered by low sample numbers in all studies thus far performed. Since the instructions provided by the company state that the i-CHROMA^{TM} device can be used to detect CRP concentrations up to 300 mg/L, measurements within this range should be reliable. Importantly, high CRP levels play a significant role in the decision to prescribe antibiotics. UK guidelines state that CRP concentrations of > 100 mg/L require an immediate antibiotics prescription for adults [11]. Therefore, the aim of the present study was to compare the i-CHROMA^{TM} POCT-system with routine turbidimetric methods based on the Architect and the ABX system using patient samples with CRP levels above 100 mg/L.

2. Material and methods

In order to compare POC and classical clinical chemistry methods, 199 anonymised patient samples (104 male and 95 female patients aged from 31 to 96 years) were investigated using the Architect ci8200SR, ABX Micros CRP 200 and the i-CHROMA^{TM} system. The blood samples were selected based on a CRP concentration of more than 100 mg/L as measured by the Architect ci8200SR. All samples met the pre-analytical standards, which means that the blood drawing was performed according to a standard operating protocol and were measured within 24 h after blood withdrawal. The analysis on the ABX and on the i-CHROMA^{TM} device were performed by the same person for all samples to reduce errors induced by differential handling over time. Blood collection (EDTA samples) was performed once for all three measurement devices as part of the routine diagnostics and no additional samples were taken for this study. For blood collection, the EDTA-system from Sarstedt (Nümbrecht, Germany) was used.

The CRP-levels on the Architect ci8200 were detected using serum samples and the method is based on a turbidimetric assay. EDTA-whole blood was used for the test procedure with the i-CHROMA^{TM} and ABX. The ABX also works with a turbidimetric assay system and the i-CHROMA^{TM} is based on a fluorescence sandwich immunoassay. The values from the i-CHROMA^{TM} were used corrected for the haematocrit values as described by the manufacturer. To perform the haematocrit corrections, the CRP-values of samples with a decreased or increased haematocrit value were multiplied with the provided factors. Haematocrit values between 37% and 42% were regarded as normal. In addition, uncorrected values were taken into consideration, since haematocrit corrections will not be possible at most POC-settings [8]. The haematocrit values were determined as a percentage of the EDTA whole blood using the Advia® 2120i device from Siemens. The used equipment was serviced as instructed by the manufacturer's recommendations. Calibrations of the Architect and the ABX were performed every day. The i-CHROMA^{TM} is self-calibrating and does not require external calibration.

Sample-specific data, such as the sample number, the CRP-value of the Architect ci8200, the day of the measurement, the day of the week and the haematocrit value were recorded. In addition, patient information about gender, age, medication and clinical diagnosis were collected. Since this study was performed as part of the routine analysis, no ethics board approval was required. The study design follows the guidelines of the Helsinki Declaration.

2.1. Statistical analysis

All 199 data sets were used for the statistical evaluation. However, samples with a concentration above 230 mg/L were not considered in the results of the ABX, since these exceed the measuring range provided by the company. The statistical evaluation was performed by the program Microsoft Excel (Redmond, USA), as well as by SPSS (Armonk, USA). The Student's T-test and the Wilcoxon test from SPSS as well as applications such as mean value, standard deviation and correlation-coefficients from Microsoft Excel were used for this purpose. Since the first data analysis showed that the measurements with the i-CHROMA^{TM} led to 2 clearly defined clouds, potential correlations between the results and measurement-induced factors such as the time and weekday of analysis, the day of measurement and the sample number of the respective day were determined. In addition, patient-and sample-related factors such as gender, age, medication and haematocrit value, were taken into consideration.

3. Results

The results of the Architect ci8200 and the ABX showed significant differences when compared to the results of the i-CHROMA^{TM} (i-CHROMA^{TM} vs. Architect ci8200 $p < 0.001$ and i-CHROMA^{TM} vs. ABX $p < 0.001$) with haematocrit corrections. In addition, the values

of the Architect ci8200 and the ABXs were also significantly different ($p = 0.001$). Nevertheless, the most pronounced differences were seen when comparing the i-CHROMA™ with the Architect ci8200 with detected differences of up to 147.6 mg/L. Comparing the values of the i-CHROMA™ with the ABX resulted in differences of up to 92.7 mg/L. The lowest maximal difference of 39.4 mg/L is seen when comparing the results of the Architect ci8200 and the ABX. These comparisons are shown in Fig. 1. The correlation coefficient of the results of the i-CHROMA™ with the Architect ci8200 was 0.4980 (Fig. 1A). The results of the i-CHROMA™ compared to those of the ABX resulted in a correlation coefficient of 0.3964 (Fig. 1B). In both cases, a clear formation of two data clusters was observed. However, the CRP measurements performed using the ABX and the Architect ci8200 shows a very high correlation coefficient of 0.9311 (Fig. 1C). These differences are also illustrated by the regression line gradients shown for each figure. The reliability of the ABX-measurements decreased for enhanced CRP-levels (>200 mg/L), which becomes clear by a flattening of the curve (Fig. 1C).

The formation of the data clusters is of interest, since this indicates a systematic error which is present in approximately 50% of the measured samples. Differences due to sample properties can be excluded as reason for the observed clustering, since the ABX and the i-CHROMA™ measurements were performed from exactly the same samples and within a timeframe of 1 hour. Further analyses were carried out to determine whether there is a defined factor responsible for the cloud formation. Both the day-to-day performance, the day of the week or the day of the project, as well as the patient-specific information (gender, age, medication and disease) were considered. None of these parameters could explain the formation of the two data clouds.

The omission of haematocrit corrections, as suggested by Brouwer and Van Pelt [8], did not improve the correlation between measurements, indicating that these corrections do not improve or worsen the data quality. The correlation coefficient of the i-CHROMA™ and the Architect was 0.5245 and for the i-CHROMA™ and the ABX 0.4191 (Fig. 2A and B) after omitting the correction.

4. Discussion

The aim of this study was to determine whether CRP values above 100 mg/L measured by the i-CHROMA™ POCT system are conform to the values measured on the Architect ci8200 or ABX. The statistical evaluation showed that the CRP values of the 3 devices are significantly different. Even though the statistical difference between the Architect and the ABX measurements are of no biological or medical relevance. Differences between the measured CRP-values can be explained by the different methodologies used. Unfortunately, the exact methods are not available for each system, which makes it difficult to compare. The following measurement-related factors could also be excluded for this variation: day of the week, day of measurement or sample number on each respective day. In addition, the following sample-related factors could be excluded: medication, disease, gender and haematocrit value.

Factors that could also influence the measurement are autoantibodies, such as anti-nuclear antibodies or the rheumatoid factor, which are detected by fluorescence. However, it is unlikely that half of the patients have such autoantibodies. In addition, in patients with systemic lupus erythematosus and in patients treated with tocilizumab, incorrectly low values for the CRP have been measured [12]. However, the treatment of tocilizumab or a systemic lupus erythematosus was not reported in any of the 200 patients. Christopolous et al. [13] describe in their paper a significant correlation between CRP and IgM antibodies against the strains urease C and urease F of the *proteus mirabilis*. Possibly, this could have an impact on the CRP-analysis. However, also in this case it is highly unlikely that half of the population was affected. In addition, the presence of high levels of triglyceride could affect CRP-analysis. Unfortunately, the removal of triglyceride with a commercially available kit resulted in a complete depletion of CRP. With hindsight, this effect was found to be previously reported for a number of different parameters including CRP [14].

In the course of the experiments for this work, it was observed that the size of the sample droplets, which are placed onto the test field, might influence the results. Smaller amounts of blood sample in the test field yielded lower results and larger amounts of liquid gave higher results. However, the drop size was not documented, because this factor should not affect the measurements when using the i-CHROMA™ POCT system and is also not described by the manufacturer as such. The relatively complicated pre-analytical handling needed to perform this analysis has also previously been reported as a potential error source [8]. In our study, one trained biomedical

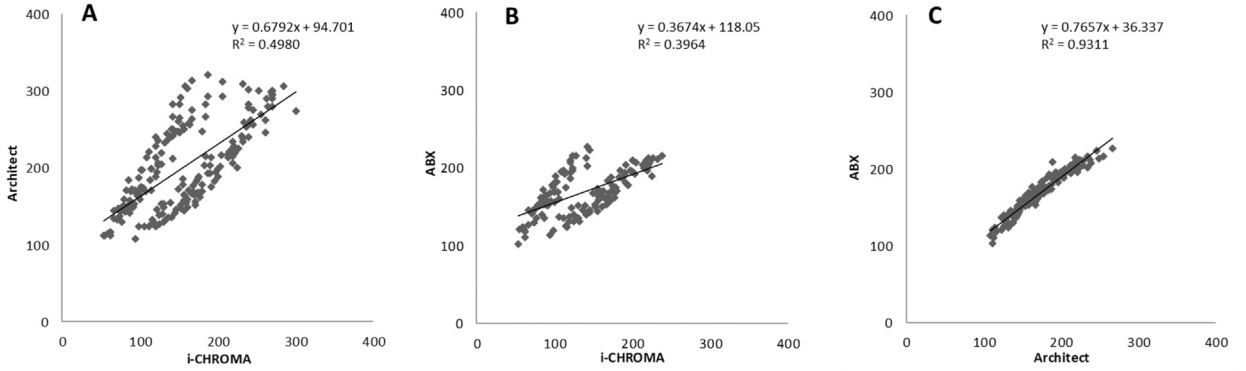

Fig. 1. Comparison of CRP-values (mg/L) analysed with the different devices. Comparison of the results of the i-CHROMA™ versus Architect with a correlation coefficient of 0.4980 (A). i-CHROMA™ versus ABX with a correlation coefficient of 0.3964 (B). Comparison of the Architect and ABX with a correlation coefficient of 0.9311 (C).

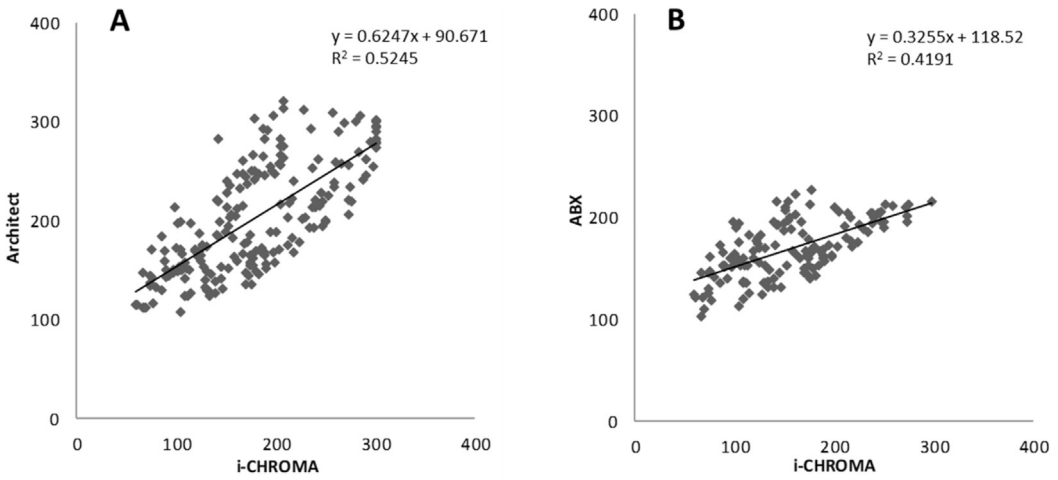

Fig. 2. Comparison of the results for CRP-levels (mg/L) of the Architect and ABX with the results of the i-CHROMA™ without the correction of the haematocrit. The correlation coefficients are 0.5245 for the comparison of the i-CHROMA™ with the Architect (A) and 0.4191 for the i-CHROMA™ and the ABX (B).

scientist performed all analysis. It is likely that the discrepancy between the measured levels increases even further when untrained staff applies such POC methodologies. Therefore, it is possible that the i-CHROMA™ test system is not suitable for the detection of higher concentrations of CRP in human blood. This observation is supported by the fact that the fluorescence immunochromatographic method was originally designed for the detection of low levels of CRP with a linearity up to 100 mg/L [15]. In the original study, the authors also included a 10-min incubation period to allow for an optimal antibody-antigen binding [15], this step is not included in the manufacturer's description of the POC-analysis. This information should be added to the product information sheet or non-comparable values should only be shown as "out of range" (>100 mg/L).

Oh et al. [6] compared the CRP results of the i-CHROMA™ with those of the TBA 200FR (Toshiba Medical Systems Corporation) and BNII (Siemens Healthineers) devices for 143 samples. The correlation of i-CHROMA™ with TBA200FR is 0.988 and with BNII 0.989. These results are by far better than the correlations of 0.4980 and 0.3964 described in the presented study. However, Oh et al. only used CRP concentrations below 50 mg/L, which explains in part the correlations found. Markovic et al. [7] compared the i-CHROMA™ with an established immune nephelometric method. With a correlation of 0.99, the 104 results of both methods fit very well. Unfortunately, only CRP concentrations below 3 mg/L were used in this study.

Brouwer and Van Pelt [8] compared 8 commercially available POCT devices. The i-CHROMA™ was also included. In this study, the results of the i-CHROMA™ were not corrected for the haematocrit; it was argued that this correction would also not be possible in a normal POC setting. The CRP concentration in the samples used for the study by Brouwer and Van Pelt was 5–200 mg/L and the results showed that the i-CHROMA™ measured at least 20% lower than the reference method for all 100 samples [8]. As a reason for this correlation, the complex pre-analytical handling needed for the analysis with the i-CHROMA™ device was mentioned. It is almost impossible to connect the capillary to the buffer tube without bringing liquid to the outer side of the tube. This problem was also noticed in the experiments performed in the presented study. In the graphs of Brouwer and Van Pelt it can clearly be observed that the deviations mainly occur when testing high concentrations of CRP with the i-CHROMA™, unfortunately, the number of high-level CRP samples was low [8]. Ciftci et al. [9] found a correlation of > 0.98 when comparing 96 CRP concentrations measured with an i-CHROMA™ device and compared with IMMAGE 800 (Beckman Coulter Inc., USA). The concentrations ranged from 4.4 to 300 mg/L, the low sample number with a bias towards tower CRP levels strongly affects the presented correlation in this study [9].

The measured data were also placed in the light of the recently published UK guidelines for antibiotic-administration in adults [11]. When the analysis would have solely been based on the values from the i-CHROMA™, a total of 39 patients (19,6%) would not have received immediate antibiotic treatment, although their CRP values were above 100 mg/L when analysed by the Architect ci8200 or ABX. Since there are no real gold-standards available for the analysis of CRP, which in itself is a problem, practitioners using this method should be aware of the negative bias of the i-CHROMA™ method need to be informed on the differences between CRP measurement methods as such.

Therefore, the present data show for the first time that high CRP-levels cannot be analysed in a reliable manner using an i-CHROMA™. Moreover, the findings support at large those described in the literature. This shows that the i-CHROMA™ CRP device should not be used for the detection of CRP levels above 100 mg/L in order to ensure patient safety.

5. Conclusion

The measured CRP concentrations of the i-CHROMA™ strongly differ in the higher CRP concentration range when compared to the measurements performed on routine diagnostic equipment. The reason for this discrepancy is currently unknown and needs further

investigation. In addition, the correction for haematocrit levels should be discussed for POC settings. The significantly reduced CRP measurements can strongly affect patient safety and result in wrong diagnostic measures.

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Conflict of interest

The authors have no conflict of interest to declare.

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