J KAU: Med. Sci., Vol 7, No. 2, pp. 45-59 (1419 A.H. / 1999 A.D.)

Comparison of Three Commercial Assays for the Determination of Serum C-Reactive Protein

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ABSTRACT. C-reactive protein (CRP) is one of the acute phase reactants produced by hepatocytes following trauma. Serial measurement of serum CRP is reported to be valuable in monitoring patients admitted to the intensive care unit (ICU). This report compared the use of three commercial assays for the routine determination of serum CRP. Two of the assays were quantitative (Tina-Quant (TQ) and Abbott TDx) whereas the other assay was semi-quantitative (Mercia latex agglutination). The effect of lipaemia on the measurement of CRP by immunoturbidimetry was also examined. Serial serum samples (n=97) from 15 patients admitted to the ICU with multiple fractures (n=7), myocardial infarction (n=4), hepatorenal failure (n=1), haematemesis (n=2), and pancreatitis (n=1) were analyzed for CRP concentrations. Good correlation (r^2=0.96) was obtained between the TQ and TDx assays in contrast to that obtained with Mercia (r^2=0.56 and 0.54, respectively). TQ assay gave significantly (P<0.005) lower CRP results than that of TDx. Lipaemia resulted in ambiguous CRP results by immunoturbidimetry, the effect being due to optical interference rather than to lipid micelles binding to CRP molecules. In conclusion, the Mercia method was labor-intensive and time-consuming, whereas the TQ and TDx assays were fast and easy to perform; however, in contrast to Mercia, required dedicated expensive equipment. Interference by lipaemia was eliminated by serially diluting the sample or by ultracentrifugation. Determination of CRP in serial samples from intensive care patients was useful in early detection of sepsis.

Keywords: C-reactive protein, Acute phase response, Lipaemic serum.

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Accepted for publication: 30 May 1998. Received: 17 March 1997.

Introduction

C-reactive protein (CRP) was described in 1930 as a substance present in sera of acutely-ill patients which bind the C-polysaccharides of the cell wall of *Streptococcus pneumonia*[1]. It was not until 1941 that it was found to be a protein and was termed "CRP"[2].

CRP is one of the positive acute-phase proteins produced by hepatocytes[3]. The synthesis of CRP is induced by interleukins - 1 and 6 and by tumour necrosis factor[4]. The measurement of serum CRP has been used in the clinical assessment of the acute phase response[5]. Elevated circulating levels are observed following tissue injury, infection, inflammation, and proliferation of malignant cells[6,7]. Furthermore, serial measurements of serum CRP has been used for monitoring therapy to inflammation and in disease progression such as in rheumatological disorders[8] and in myocardial infarction[9]. Serum CRP concentrations were found to be significantly lower in intensive care patients without any evidence of infection as compared with those with clinical infection[10]. This finding suggests a useful role for serial measurement of CRP in serum in the early detection of sepsis.

The identification and purification of CRP has allowed production of antibodies and the establishment of a number of sensitive and specific immunoassays for its measurements. A number of assays are commercially available. They are based on radial immunodiffusion, immuno-turbidimetry, rate nephelometry, homogenous enzyme immunoassay, and immuno-turbidimetry of fluorescence polarization[11]; however, it is the last two that are frequently used in routine practice.

Lipaemia is known to interfere with immuno-turbidimetric assays, producing low or ambiguous values[11]. In the case of CRP, such interference can either be due to CRP binding to lipid micelles making it inaccessible to binding to CRP antibodies, or to interference with the optical detection on the end point. This study examined both possible causes of interference.

Using sera from patients admitted to the intensive care unit at our hospital, this study compared the use of three different immune-based commercial assays in the routine determination of CRP. Two of the assays were designed for quantitative measurement of CRP whereas the other assay was for either screening purposes or for semi-quantitative CRP measurement. The study assessed the value of accurate measurement of serum CRP.

Patients and Methods

Patients: Serial serum samples were obtained from 15 patients with a variety of disorders (see Table 1 for classification) who were admitted to the Intensive Care Unit (ICU) at the King Khalid National Guard Hospital (KKNGH), Jeddah, Saudi Arabia, were used to collect serial serum samples. Serum samples were collected at admission

and at 6, 12 and 24 hours, and daily following admission until discharge from the ICU. Ethical Committee approval was obtained. Samples were stored at -20°C until analysis.

TABLE 1. Classification and respective number of patients investigated.

Diagnosis	Number of Patients
Multiple injuries	7
Myocardial infarction	4
Hepato-renal failure	1
Haematemesis	2
Pancreatitis	1
TOTAL	15

Methods: Serum CRP levels were determined using three different commercial assays. The assay kits (Mercia, TDx Abbott CRP, and Tina-Quant (TQ)) were obtained from Mercia Diagnostics (Guilford, Surrey, United Kingdom), Abbott Diagnostics (Abbott Park, Illinois, U.S.A.), and Boheringer Mannheim (France), respectively. The Mercia assay, designed for screening purposes and/or semi-quantitative analysis, was performed on an agglutination plate whereas TDx and TQ assays, designed for quantitative analysis, were performed using an Abbott TDx machine and a Hitachi 717 auto-analyser, respectively. The TQ assay was calibrated using a Precimat CRP calibrator (Boehringer Mannheim) containing CRP at 86 mg/l. The TDx assay was calibrated using TDx CRP calibrators (6 vials) containing CRP at 0, 40, 80, 120, 180, and 260 mg/l. Imprecision studies for both the TDx and the TQ quantitative assays were performed. Procedures for all assays were according to the manufacturer's instructions. Analysis was performed in the Department of Clinical Chemistry, King Abdulaziz University Hospital, Jeddah.

The effect of lipaemia on the determination of serum CRP by immuno-turbidimetry was investigated as follows: A grossly lipaemic sample (triglyceride level=28.0 mmol/l and three non-lipaemic samples with low (2 mg/l) and high (338 and 390 mg/l) CRP values as determined by immuno-turbidimetry using Technicon CRP assay (Technicon Diagnostics, Basingstoke, Hants., U.K.) were collected retrospectively. The assay was performed using the Kone (Specific) auto-analyser (Labmedics, Ltd., Stockport, Cheshire, U.K.). The analysis was performed in the Department of Clinical Chemistry, Royal Liverpool University Hospital, U.K. Procedures were according to the supplier's instructions.

Serum samples with high CRP (390 mg/l) were serially diluted in the grossly lipaemic sample and final dilutions assayed for CRP concentration. CRP determination was performed in five replicates in all investigations. The grossly lipaemic sample was serially diluted in a low CRP (2 mg/l) sample. To 100 µl aliquot of each dilution, 100 µl of a high CRP (338 mg/l) sample was added. Samples were vortexed and incubated for one hour at room temperature before being assayed for CRP concentration. The effect

of ultracentrifugation on lipaemia interference was examined as follows: The grossly lipaemic sample (800 µl) was mixed with a high CRP (338 mg/l) sample (800 µl) and incubated for one hour at room temperature. An aliquot (100 µl) was taken for CRP estimation prior to ultracentrifugation at 80,000 xg for 30 minutes using an MSE Superspeed 65 (MSE, Crawley, Sussex, U.K.). An aliquot (800 µl) of high CRP sample was also centrifuged to examine the effect of centrifugation. Aliquots were carefully withdrawn and CRP concentration determined.

Royston's development of the Shapiro-Francia W test for normality[12] showed that CRP data obtained in all three assays differed significantly from a normal distribution. Wilcoxon's non-parametric test was, therefore, used for statistical analysis of data.

Results

Serial serum samples (n=97) from 15 patients were analyzed for CRP concentrations using three different commercial assays. CRP was detectable in most samples by all three assays (97.9% using TQ, 91.9% using TDx, and 91.5% using Mercia assays). There was good correlation between CRP results obtained by the TQ and by the TDx assays (r^2=0.96) (Fig. 1) whereas the correlations between CRP results obtained by the TQ and the Mercia (data not shown) and between the TDx and the Mercia assays (Fig. 2) were poor (r^2=0.56 and 0.54, respectively).

Serum CRP values obtained by the TQ assay were significantly lower than those obtained by the TDx assay and the Mercia assay (P<0.005 and P<0.005, respectively). Furthermore, CRP values obtained by the TDx assay were significantly (P<0.005) lower than those obtained by the Mercia assay.

Imprecision ranged from 4.5, 1.4, and 1.0% at 9.17, 109.57, and 302.2 mg/ml in the TQ assay and from 10.6, 2.7, and 2.0% at 2.03, 11.81, and 39.4 mg/ml in the TDx assay, respectively in each case.

In 11 patients, serial serum samples showed increasing CRP concentrations following admission to the ICU. Figure 3 shows CRP response in serial samples from patients admitted to the ICU with: a) rib fractures following a road traffic accident, b) myocardial infarction, and c) major burns and multiple orthopaedic fractures. In patient A), serum CRP increased steadily, reaching a peak at 100 hours following admission. Although results obtained by all three assays were parallel, the pattern obtained by the Mercia assay was a stepwise fashion (Fig. 3a). In patient b), serum CRP concentrations were elevated at admission. Increasing CRP levels were observed when using the TQ and the TDx assays whereas no change in CRP concentration was obtained from the Mercia assay (Fig. 3b). Figure 3c shows CRP response in a patient admitted with multiple fractures and burns. CRP concentrations were elevated at admission and declined for two days prior to a subsequent rise at 95 hours following admission. This increase in CRP concentration followed a surgical procedure 40 hours previously. The CRP level declined for one day before starting to rise again. This latter rise was attrib-

uted to microbiological evidence of sepsis two days later. Values obtained by the TDx and TQ assays were similar compared with an exaggerated response obtained with the semi-quantitative method, Mercia.

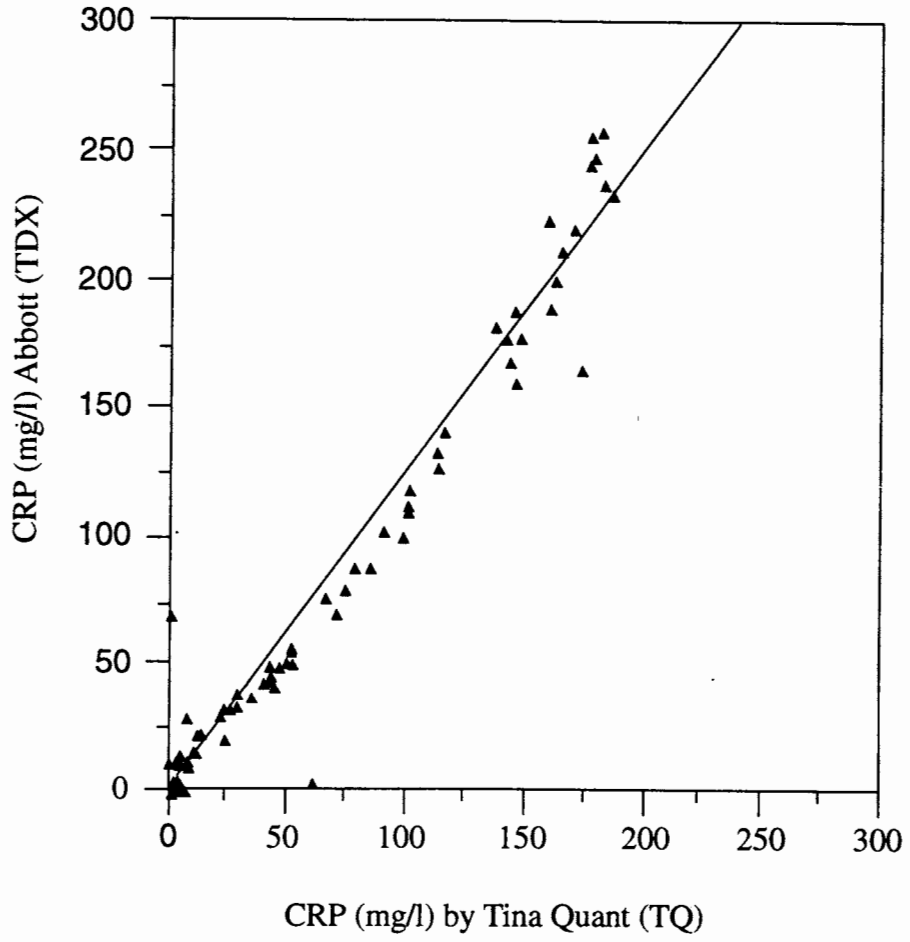

Fig. 1. Correlation between serum CRP values obtained by the TQ immuno-turbidimetric method and by the TDx fluorescence polarization method.

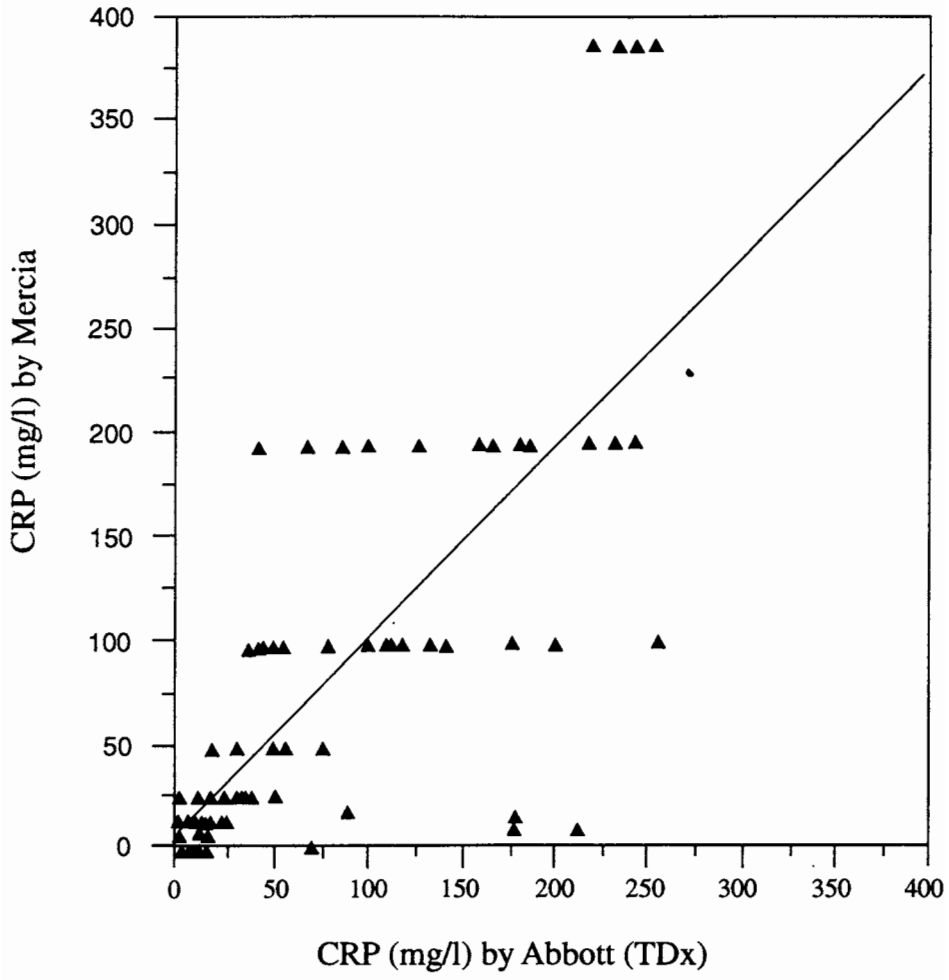

Fig. 2. Correlation between the serum CRP values obtained by the TDx assay and by the Mercia semi-quantitative latex agglutination assay.

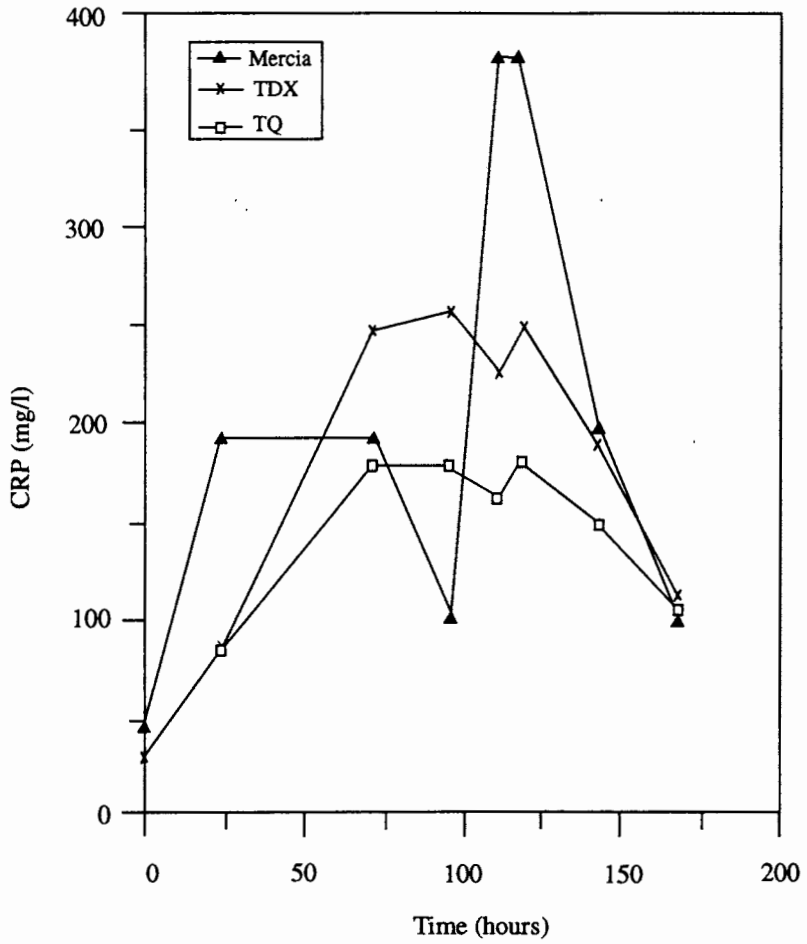

Fig. 3a. Serum CRP concentrations in serial samples collected from patients admitted to the intensive care unit with multiple fractures following a road traffic accident.

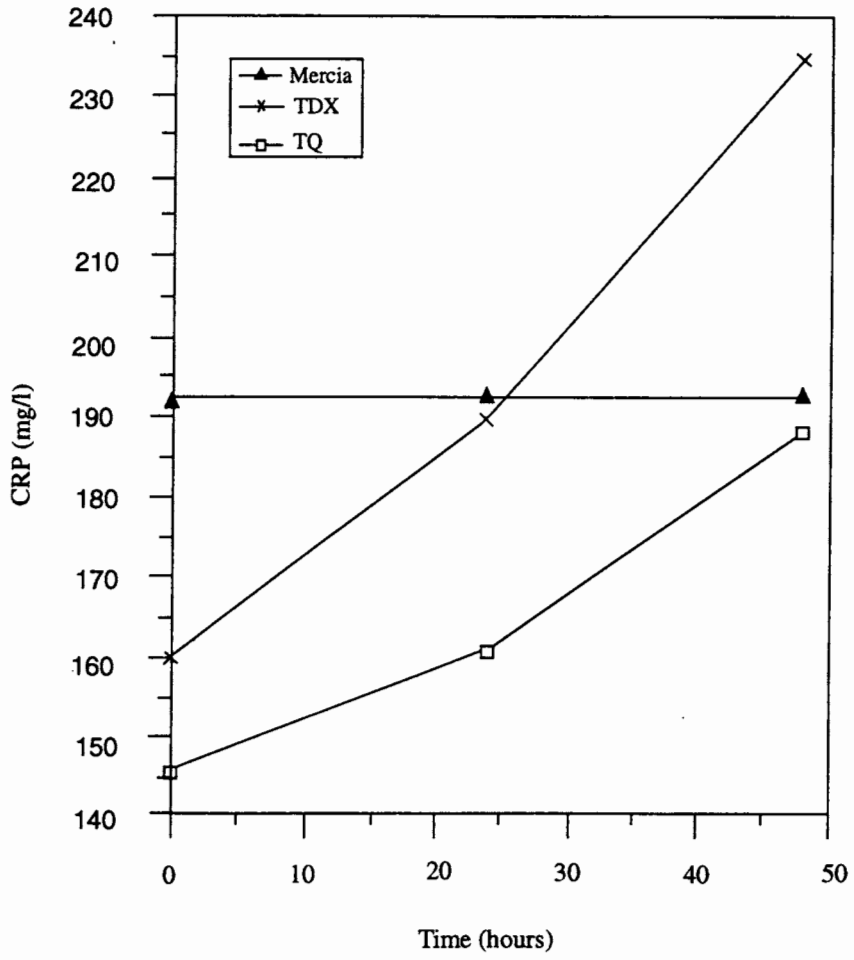

Fig. 3b. Serum CRP concentrations in serial samples from a patient admitted to the intensive care unit with myocardial infarction.

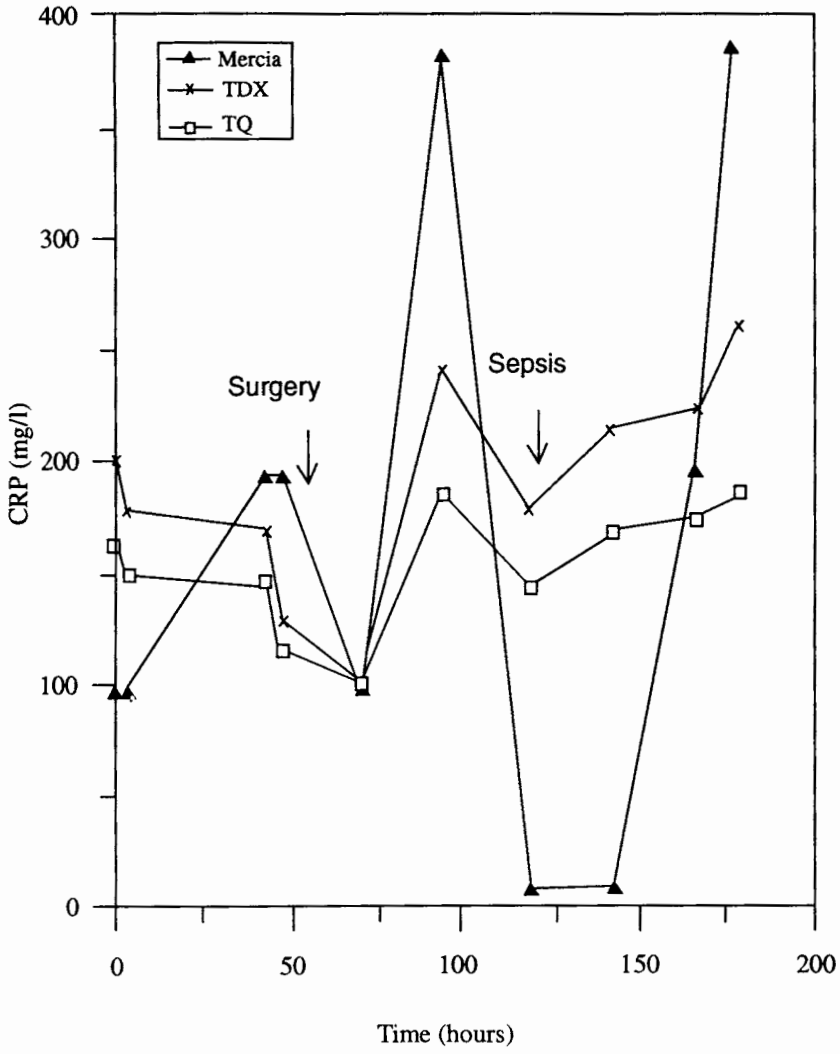

Fig. 3c. Serum CRP concentrations in a patient admitted to the intensive care unit with major burns and multiple orthopaedic fractures. A marked increase in CRP concentration was observed following a surgical procedure and prior to microbiological evidence of sepsis.

The effect of lipaemia on the determination of CRP was investigated. A grossly lipaemic sample was diluted serially with a normal serum sample containing low CRP (2 mg/l). This diluted out the lipaemia. To these dilutions, 100 µl serum samples containing 338 mg/l CRP were added. CRP was not significantly recovered (<19%) until a dilution of 1:16 was achieved (recovery 78%). 100% recovery was obtained at dilutions higher than 1:32 (Fig. 4). A sample with a high CRP concentration (390 mg/l) was serially diluted in a grossly lipaemic sample. CRP was detected at all dilutions; however, values did not dilute out as expected (Fig. 5) and remained constant. Furthermore, recovery was less than 38%. The possibility that CRP was bound to lipid micelles resulting in apparently low CRP was examined by spiking a grossly lipaemic sample with a high CRP sample (338 mg/l) following ultracentrifugation. An aliquot was assayed for CRP activity; 80% of added CRP was recovered.

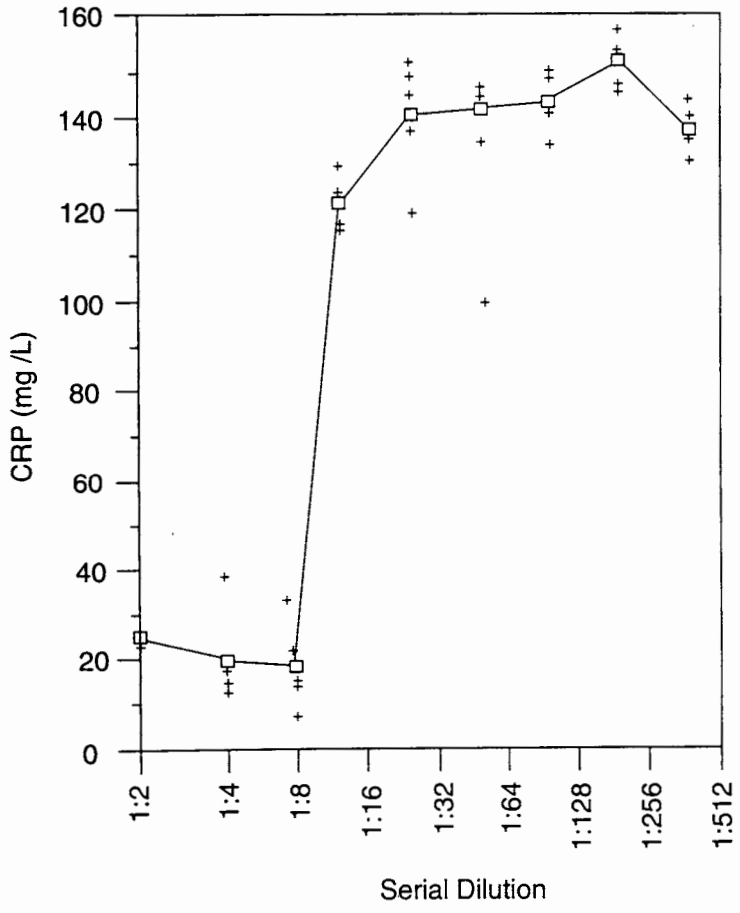

Fig. 4. CRP concentrations obtained following spiking of a grossly lipaemic sample that had been serially *diluted with* a low CRP sample (2 mg/l). Added CRP was recoverable when lipaemia was diluted higher than 1:8, reaching expected value (. . .) at dilutions higher than 1:32.

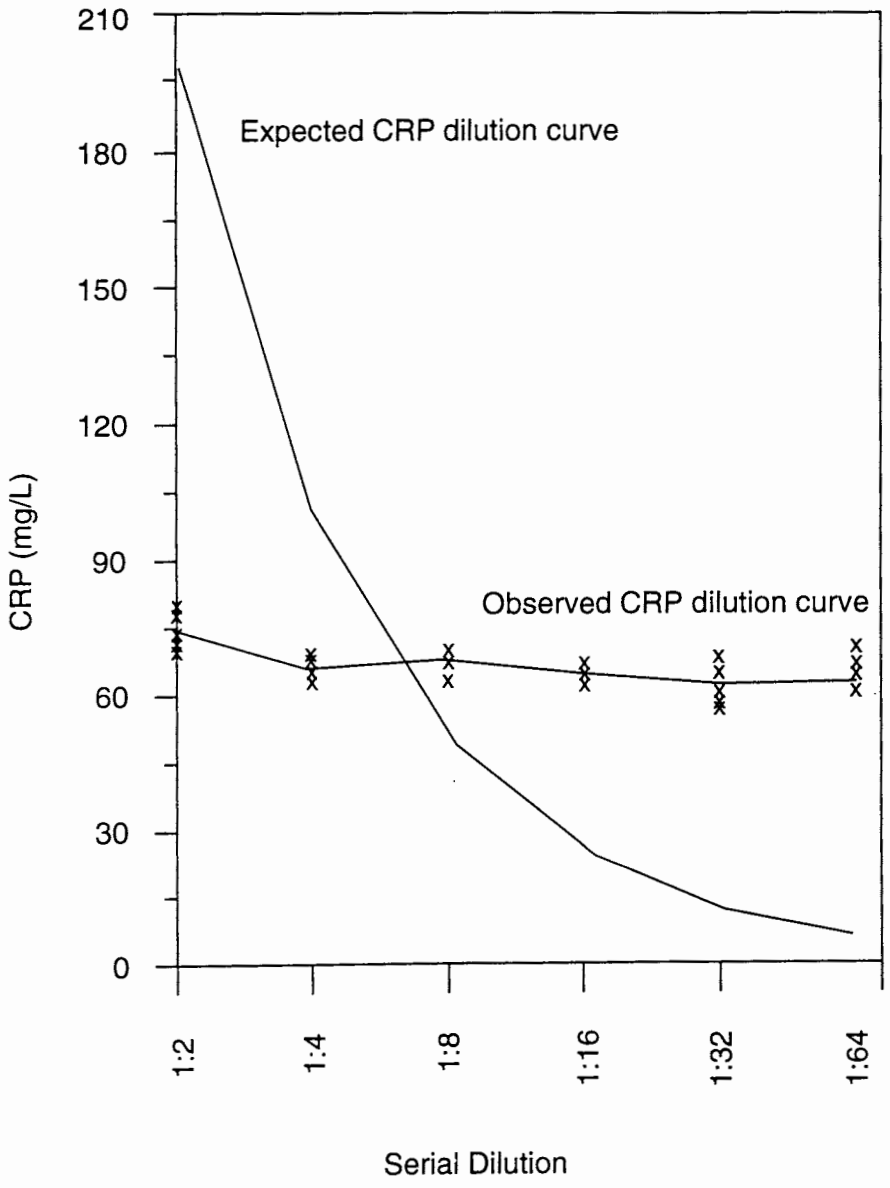

Fig. 5. CRP concentrations when serially diluted in a high CRP sample (390 mg/l) in a grossly lipaemic sample. Ambiguous response with only 38% activity of recoverable was observed. The expected serial dilution response was not obtained.

Discussion

This study examined three different commercial assays for the determination of ser-

um CRP in patients admitted to the ICU for various reasons. CRP levels were detectable in most samples (>91.5%) by all three assays. There was good correlation between CRP values obtained by the two quantitative methods (TQ and TDx) (r^2=0.96) whereas the correlation with the semi-quantitative Mercia assay was poor, if any.

The Mercia CRP assay was designed for quantitative and semi-quantitative analysis of serum CRP. It employs direct latex agglutination technique and the stated detection limit is 6 mg/l. The assay is ideal for screening purposes where it takes less than five minutes to obtain a result. The assay requires a small sample volume (50 µl) which makes it suitable for paediatric sample screening. However, when using the assay for semi-quantitative analysis, serial dilutions of patients sera are required and, as in the quantitative mode, inclusion of positive and negative controls is required. CRP titre is recorded as the greatest dilution showing a clear positive agglutination. A conversion of the titre to an approximate concentration is made by multiplying the titre with the detection limit of the assay (6 mg/l). This is very crude as it relies on the choice of the end point which may vary between different operators and any such discrepancy would at least lead to a two-fold difference in reported CRP concentrations. Furthermore, the higher the dilution, the greater the possibility of erroneous CRP results. However, for all practical purposes, high CRP titre implies a markedly elevated CRP.

The measuring range of the quantitative TQ assay was stated as being 3-110 mg/l. The manufacturer claims interference by lipaemia up to 26 mmol/l and we were not able to obtain CRP results on a sample with triglycerides concentration of 28.1 mmol/l.

The TDx assay utilizes fluorescence polarization immunoassay technology. Results obtained by this assay correlated well with those obtained with the TQ immunoturbidimetric assay. The assay was fast and easy to use; however, it was best used for batch analysis of samples. This makes it suitable for emergency "out-of-hours" analysis. Furthermore, as in the case of the TQ assay, automated expensive equipment was required.

The effect of lipaemia on the measurement of CRP was examined. CRP in non-lipaemic samples diluted as expected both in saline and in a low CRP sample (data not shown); however, when diluted in a grossly lipaemic sample, only 38% of the CRP concentration was recoverable. The fact that no dilutional effect was observed invalidates the results obtained. The effect of lipaemia was further examined by diluting the lipaemia in a low CRP sample followed by spiking the dilutions with a high CRP sample. CRP concentration was detectable at dilutions higher than 1:32. This finding suggested either that: a) lipaemia interfered with the optical measurement of immunoturbidimetry or b) lipid micelles bound CRP and that at high dilutions, the binding moiety is decreased and binding capacity exceeded, resulting in increased recoverable CRP. To distinguish between these two possible causes, the grossly lipaemic sample was incubated with a high CRP sample and subjected to ultracentrifugation. A carefully aliquoted sample showed CRP activity of about 90%, suggesting that CRP did not significantly bind to lipid micelles. This suggested that interference with CRP meas-

urement in lipaemic samples is predominantly due to lipaemia interfering with the optical measurement of the endpoint of immunoturbidimetry. It is suggested that in order to eliminate interference by lipaemia in immunoturbidimetric assays, all lipaemic samples should be serially diluted in a serum sample with a low CRP concentration or saline or, if available, to ultracentrifuge.

Another valuable reason for the determination of serum CRP is in the investigation of the acute abdomen[22], where patients being investigated for pancreatitis who had symptoms for more than 24 hours, had CRP concentrations greater than 10 mg/l in more than 80% of the cases[23].

It has been reported[24] that daily monitoring of patients admitted to the major injury unit at the Birmingham Accident Hospital (U.K) showed that the survivors had significantly lower CRP values than that obtained by non-survivors. Although this study was not aimed at studying the CRP response by different patient groups, the range of CRP concentration observed in the ICU setting was used to study the routine use of the three assays.

In conclusion, this report compared three different assays for the routine determination of CRP. Semi-quantitative assays were best for screening purposes. The report highlighted the value of an accurate and rapid CRP assay and the use of quantitative assays for routine use was therefore recommended. One of our patients showed elevated CRP levels two days prior to microbiological evidence of sepsis. This highlighted the value of serially measuring the serum CRP in patients admitted to the ICU. Lipaemia interferes with CRP determination by immunoturbidimetric assays. Such interference can be eliminated by serial dilution or by ultracentrifugation.

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مقارنة بين ثلاث طرق لتحديد بروتين سي في مصل الدم

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المستخلص. إن مادة *(CRP)* هي إحدى متفاعلات التفاعل الحاد الذي يتم إنتاجه في خلايا الكبد ويفرز في حالة الإصابة. هذا، وإن قياس *(CRP)* في الدم بصورة متتالية له أهمية في مراقبة مرضى وحدة العناية المركزة. وهذا البحث عبارة عن مقارنة عملية بين ثلاث اختبارات: اثنان منها قياسيان والثالث نوعي، ودراسة أثر ارتفاع الدهون في الدم على قياس *(CRP)*. ولقد أخذت سلسلة من عينات الدم من خمسة عشر مريضاً من وحدة العناية المركزة منهم سبعة مرضى مصابون بكسور مختلفة، وأربعة مصابون بجلطة في القلب، ومريض واحد يعاني من فشل كلوي وكبدي، واثنان مصابان بنزيف في الجهاز الهضمي، ومريض مصاب بالتهاب في البنكرياس. وظهر من الدراسة أن هناك نسبة تقارب جيدة بين الاختبارات القياسية *(r=0.96)* وغير جيدة بالنسبة للاختبار النوعي *(r=0.56-0.54)*، كما تبين أن أحد الاختبارات القياسية *(TQ)* أعطى نتائج متوسطها أقل من الاختبار القياسي *(TDX)*، واتضح أن لارتفاع الدهون في الدم أثر في نتائج *(CRP)* بصورة لا تخلو من الالتباس لأن هذا الأثر كان نتيجة التداخل في ميكانيكية القياس الشعاعية وليس نتيجة لاتحاد جزيئات الدهون مع جزيئات *(CRP)*.