The effect of different methods of storage on the results of serum total CO₂ assays

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ABSTRACT

Metabolic acidosis frequently complicates end-stage renal failure. In haemodialysis patients its severity is usually monitored by measurement of the total CO₂ (TCO₂) level. Samples from ‘satellite dialysis’ patients are often stored prior to analysis. We investigated the effect of storage of 21 samples for 24 h under different conditions prior to analysis. If samples were stored at room temperature the TCO₂ fell from 22.7 ± 4.2 mmol/l to 21.6 ± 3.7 mmol/l (P = 0.001). If the same samples were spun and stored at 4 °C the TCO₂ was 22.4 ± 3.9 mmol/l (P = not significant). We conclude that the magnitude in the fall of TCO₂ stored at room temperature for 24 h is unlikely to be clinically significant and can be prevented by spinning the sample and refrigerating it.

INTRODUCTION

Metabolic acidosis is increasingly considered to be a cause of morbidity, and possibly mortality, among dialysis patients. There is evidence that it contributes both to muscle wasting [1] and osteomalacia [2]. In the U.K., the Renal Association [3] has recommended that all chronic haemodialysis patients should have pre-dialysis plasma bicarbonate within the reference range quoted by the local laboratory after 3 months of treatment. However, the document does not provide guidance on how samples should be stored or analysed.

Plasma bicarbonate is usually measured as total CO₂ (TCO₂). This assay measures not only the bicarbonate in the sample, but also the carbonic acid and dissolved CO₂ [4]. Delays may occur in analysing samples from satellite dialysis units which do not have on-site laboratories. This delay may lead to a number of possible errors: CO₂ may be lost to the air in contact with the sample, or it may be generated by red cell metabolism. In our experience, some laboratories are reluctant to perform TCO₂ assays from satellite units owing to concerns about the accuracy of the result.

METHODS

Blood (50 ml) was obtained from 21 patients before undergoing routine haemodialysis. This was divided between five unheparinized tubes containing accelerator beads (Sarstedt, Leicester, U.K.). The tubes had a total volume of 15 ml, but were filled to the 10 ml ‘fill line’ as accurately as possible by eye. The screw caps were fastened, leaving the blood in contact with 5 ml of air. One sample from each patient was analysed immediately and the remaining four samples were stored for 24 h as follows: sample 1, stored at room temperature (25 °C) unspun; sample 2, stored at room temperature and spun; sample 3, refrigerated (4 °C) and unspun; and sample 4, refrigerated and spun. All samples were processed within 40 min of being taken from the patient. Samples were

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analysed on an Olympus 640 analyser by the phosphoenolpyruvate catalyse method [5]. The inter-assay coefficient of variation was 5.2% at a level of 14.6 mmol/l. Informed consent was obtained from all patients and ethical approval was obtained from the local ethics committee.

Statistical analysis was performed using the Arcus software package. The differences between group 1 and each of groups 2–5 were tested using Bonferroni multiple comparisons, where \( P \) values < 0.0125 were considered significant. This statistical test was used to avoid the increased risk of a type I error associated with multiple \( t \) tests using the same baseline value. The 95% limits of agreement were calculated by the methods described by Bland and Altman [6].

**RESULTS**

The results of the study are shown in Table 1. Two-way ANOVA showed a significant difference between the groups \( (P = 0.0028) \). The results demonstrate that the TCO\(_2\) of samples unspun and stored at room temperature falls by a small, but statistically significant, amount. If the sample was spun and cooled then there were no significant changes. However, if the sample was only spun or cooled then the inaccuracy of the result persisted.

**DISCUSSION**

If the recommendations that are set out in the Renal Association Standards document [3] are to be fulfilled then the techniques for storing and analysing samples must be defined and standardized. This is particularly important if the results from different units are to be audited comparatively. The process and aims of audit will be undermined if this methodology is not established. In the present study, the change in TCO\(_2\) which occurred if the sample was stored for 24 h at room temperature was small, with a mean of 1.1 mmol/l. We do not consider this to be of significance in routine clinical practice. Furthermore, we do not believe that the size of these errors precludes samples from satellite dialysis facilities being analysed for TCO\(_2\) at a remote laboratory. We have shown that this error can be eliminated, but only if the sample is spun and refrigerated prior to storage.

Two other studies have looked at the storage and transport of blood samples prior to TCO\(_2\) measurement. Bray et al. [7] found that when 12 samples were stored uncapped for 24 h, the mean TCO\(_2\) fell from 23.1 ± 0.3 mEq/l to 20.4 ± 0.4 mEq/l \( (P < 0.05) \). As samples are rarely stored uncapped in clinical practice we chose not to evaluate this in the present study. Kirschbaum [8] compared samples from 24 dialysis patients that were either transferred to a central laboratory by aircraft following centrifugation and refrigeration for analysis the following day, or allowed to coagulate at room temperature and assayed ‘on-site’ within a few hours. The mean TCO\(_2\) for the samples assayed at the central laboratory was 17 ± 3 mEq/l, and for those at the local laboratory was 22 ± 3 mEq/l \( (P = 0.01) \) [8]. The magnitude of the change in TCO\(_2\) was much greater than in the present study and could lead to a spurious suspicion of a metabolic acidosis. In Kirschbaum’s study [8] the different sets of samples were assayed using different chemistry analysers. Although both used phosphoenolpyruvate catalyse assays, it may be that differences between the different instruments were responsible, at least, in part, for the larger difference in results. Another methodological difference between this study and our own was the use of Vacutainer tubes containing gel and clot activator. Further studies to compare the type of tubes used to store samples may produce interesting results.

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**REFERENCES**


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