Investigation of methodological sources of bias in the measurement of vitamin K_1 (phylloquinone) in human serum at endogenous concentrations

Card DJ[1], Shearer MJ[1], Ruwona P[1], Schurgers LS[2] and Harrington DJ[1].
[1]Nutristasis Unit, GSTS Pathology, St. Thomas' Hospital, London SE1 7EH, United Kingdom.
[2]Biochemistry Department, University of Maastricht, The Netherlands

Background and aims

Vitamin K is an essential micronutrient involved in the posttranslational modification of various Gla proteins including coagulation factors II, VII, IX and X, proteins C and S, osteocalcin and matrix Gla protein (Figure 1). Serum or plasma vitamin K_1 (phylloquinone) concentrations correlate with dietary intake and functional markers of vitamin K status (e.g. undercarboxylated factor II (PIVKA II)) and provide a useful guide to tissue stores. The Vitamin K External Quality Assurance Scheme (KEQAS) is dedicated to the harmonisation of vitamin K measurement, enabling more reliable cross-comparison of studies of vitamin K status. Previous data has shown that assay bias contributes to about 30% variance seen in comparison of inter-laboratory vitamin K_1 analysis.

The aim of this study was to quantify the assay bias for each KEQAS participant and to attempt to link the data to specific methodological variations.

Methods

Sources of bias were investigated by distributing a questionnaire to all KEQAS participants (n = 25, response rate = 100%) specifically relating to key methodological information. Bias was calculated for vitamin K_1 results returned between February 2009 and October 2012 generated from serum (n = 22) and ethanolic (n = 10) samples. During this period the majority of KEQAS participants employed HPLC with in-line chemical reduction and fluorescence detection, however two laboratories employed HPLC with mass spectrometry (HPLC-MS).

Results

Mean individual laboratory bias ranged from -28 to 30%. Laboratory bias ranking altered very little when results from ethanolic samples were removed indicating bias did not originate from chromatographic interferences in the serum. The two HPLC-MS methods returned the two most negatively biased values of -22 and -28 % respectively. A major finding was that 7 groups (28 %) using a commercially available calibrator, returned results with positive bias (range = 13 to 23 %, mean = 19%).

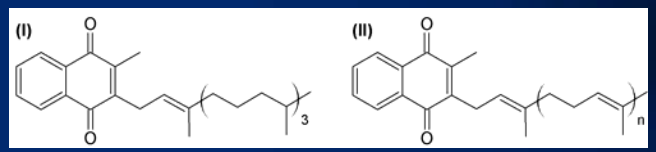

Figure 1: Vitamin K_1 (phylloquinone (I)) and vitamin K_2 (the menaquinones (II))

Conclusions

The KEQAS Steering Committee is currently in talks with the manufacturer of the calibrator in order to investigate the reasons for the disparity between KEQAS participants using this commercial calibrator and participants using calibrators prepared in-house. Currently it is not possible to determine the significance of the negative bias seen in results from the two HPLC-MS methods. This may reflect their status as an improved method, free from interferences or may be a result of other confounding methodological factors. Measurement of vitamin K_1 by HPLC-MS represents a potential reference method although further evidence is required to establish this.

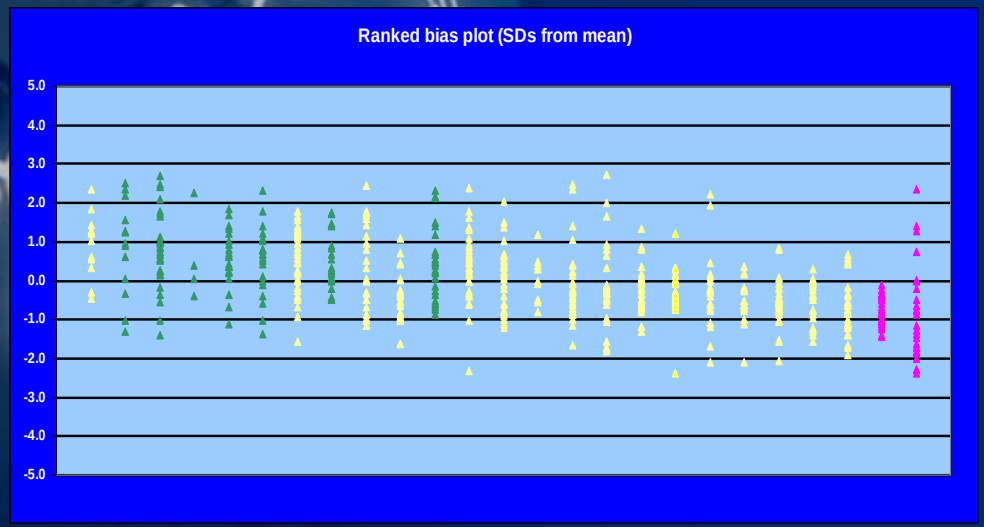

Figure 2: Ranked bias plot (green = commercial calibrator, pink = LC-MS/MS)

References

Card DJ, Shearer MJ, Schurgers LJ, Harrington DJ. The external quality assurance of phylloquinone (vitamin K(1)) analysis in human serum. Biomedical Chromatography. 2009; 23(12):1276-82.