The launch of a new pilot external quality assurance scheme for PIVKA-II determination

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The UK NEQAS for Vitamin K (KEQAS) is an international EQA provider for the measurement of Vitamin K in human serum. A new pilot scheme for the analysis of PIVKA-II (Protein Induced by Vitamin K Absence or Antagonist (undercarboxylated prothrombin)) is being launched in 2019. PIVKA-II has diagnostic value as a functional marker of vitamin K status [1] including vitamin K antagonism e.g. warfarin [2]. PIVKA-II is detectable at very low concentrations in the plasma of healthy individuals [3] and becomes elevated when there is insufficient dietary vitamin K to facilitate efficient γ-carboxylation of prothrombin or when a vitamin K antagonist inhibits the salvaging of spent vitamin K by the vitamin K epoxide reductase enzyme (VKOR). PIVKA-II is also a useful biomarker for hepatocellular carcinoma (HCC) where plasma concentrations become highly elevated independently of vitamin K status. PIVKA-II is measured in combination with alpha-fetoprotein for the detection and the monitoring of progression of HCC [4].

Introduction

PIVKA-II is an abnormal form of prothrombin (undercarboxylated prothrombin), produced when dietary vitamin K is limited, in the presence of a vitamin K antagonist e.g. warfarin or in patients with HCC. Under normal physiological circumstances, precursor prothrombin is carboxylated in the liver by vitamin K dependent carboxylase forming native prothrombin. This reaction converts the 10 N-terminal glutamic acid residues (Glu) to γ-carboxyglutamic acid residues (Gla) [3]. In the absence or antagonism of vitamin K, there is failure to convert all or some of the Glu residues, forming and releasing abnormal PIVKA-II into the bloodstream (Figure 1). Gla residues play a crucial role in the biological activity of vitamin K dependent proteins, which include coagulation factors II, VII, IX and X, proteins C and Z, osteocalcin and matrix Gla protein. Gla residues provide binding sites for calcium ions [5], resulting in an activating conformational change [6]; allowing them to bind with phospholipids in the prothrombinase complex, which consists of prothrombin, factor Xa, factor Va and phospholipids [7]. As PIVKA-II lacks Gla residues, it is unable to bind to calcium ions, remaining biologically inactive in the coagulation cascade. Plasma PIVKA-II concentrations can be utilised in the assessment of vitamin K status or antagonism. As a diagnostic marker of HCC PIVKA-II is usually utilised in combination with other markers such as alpha-fetoprotein (AFP).

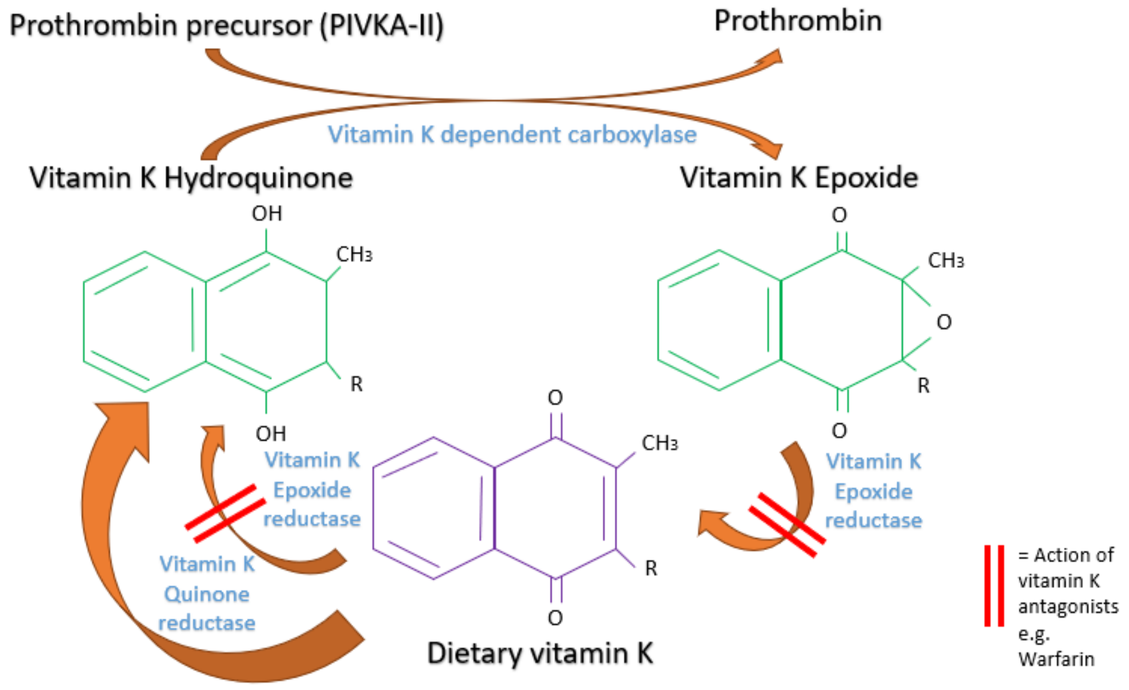

Figure 1: The vitamin K cycle. Vitamin K acts as a cofactor for gamma-glutamyl carboxylase, that converts Glu to Gla residues in prothrombin and other proteins. It is oxidised when utilised as a cofactor for the carboxylase enzyme and is then salvaged by a series of reduction reactions catalysed by the vitamin K epoxide reductase and other reductase enzymes. It In the absence of vitamin K or the presence of vitamin K antagonists (e.g. warfarin), the action of gamma-glutamyl carboxylase is inhibited, releasing PIVKA-II into the blood stream.

Plasma PIVKA-II concentrations have been shown to be elevated in patients with HCC, independently of vitamin K status, however the relationship between these factors is not well defined. It has been suggested that PIVKA-II plays a pathological role in HCC by mimicking hepatocyte growth factor [8, 9, 10]. In doing so promoting an increased rate of uncontrolled hepatocyte proliferation. Furthermore, PIVKA-II promotes angiogenesis [11, 12], increasing the likelihood of metastasis.

PIVKA-II has been shown to be a marker with high specificity and selectivity for diagnosis of and as a predictor of prognosis in HCC patients [13] (Figure 2) when used in combination with AFP [4]. Its use can be limited by pharmacological vitamin K antagonists e.g. warfarin which also cause PIVKA-II to be raised. A combination of PIVKA-II and AFP is suggested to be the best available option for laboratory diagnosis of HCC, which can help instigate further investigations e.g. scans.

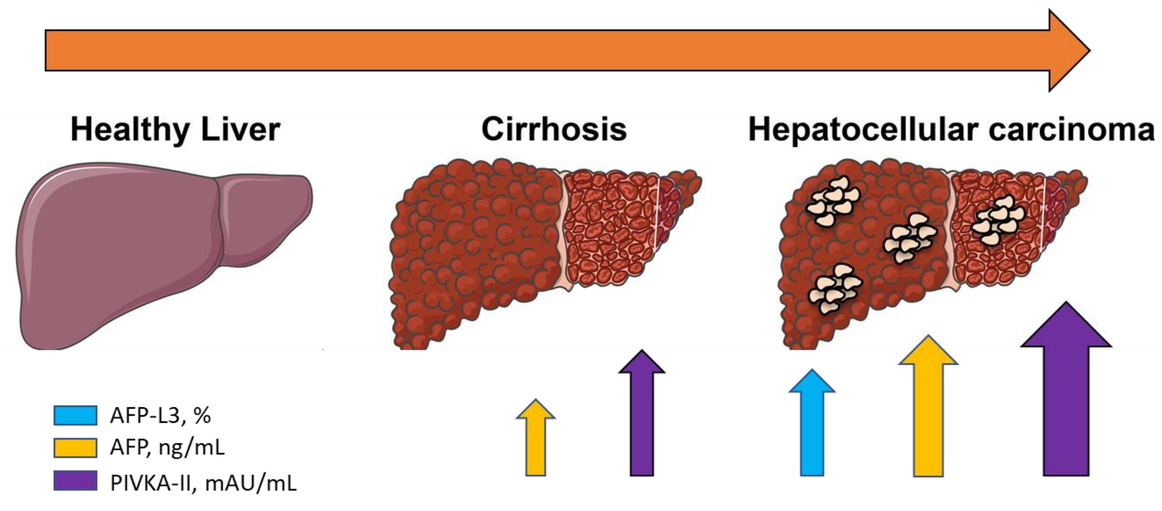

Figure 2: Biomarkers of hepatocellular carcinoma. The diagram represents data collected from Park et al., 2017 [4]. Serum samples were analysed from 77 individuals with liver cirrhosis (LC) and 79 individuals with HCC, and the median and interquartile ranges calculated, avoiding misleading mean values produced from extreme outliers. The median of serum biomarkers PIVKA-II, AFP and isoform of alpha-fetoprotein AFP-L3 were all significantly higher in HCC patients. LC results: AFP-L3, 0%; AFP, 3.7ng/mL; PIVKA-II, 21.5 mAU/mL. HCC results: AFP-L3, 10%; AFP, 93.4ng/mL; PIVKA-II, 249.0 mAU/mL.

Methods of measuring PIVKA-II

Various methods are available including automated immunoassays e.g. Abbott Architect, Fujirebio Lumipulse G 600II, immunoassays developed in-house [3] and mass spectrometry [14]. Prothrombin has ten Gla residues, and so there are potentially many different isoforms that may be present in circulation and the ratios of the isoforms may also differ depending on the clinical scenario i.e. vitamin K deficiency, vitamin K antagonism and HCC. Assays use different antibodies (e.g. C4B6, C310) and it is not currently known which circulating isoform(s) immunoassay methods detect. However it is likely that many are directed towards fully de-carboxylated prothrombin as this antigen can be synthesised conveniently in the laboratory [15], the antibodies generated may also have cross-reactivity with other circulating PIVKA-II isoforms.

Studies carried out at the UK NEQAS for Vitamin K demonstrated that antigen diluted in to pooled serum gave a recovery of >90% and was stable for 4 weeks at room temperature, indicating the suitability of the material for distribution for EQA purposes.

Discussion

Due to the uncertainties in the measurement of PIVKA-II described above, it is desirable to initiate an EQA scheme that allows the assessment and comparison of methods. The use of PIVKA-II as a biomarker has been the focus of various clinical studies utilising different analytical methods and an EQA scheme makes it possible to evaluate their comparability. Additionally, it would aid accurate diagnosis as a tool to assess the performance of individual methods.

Aims

- To improve the quality of PIVKA-II analysis used with the purpose of developing patient care
- To establish the continuity of the analytical methods used for the measurement of PIVKA-II
- To provide guidance if laboratories are out of consensus with other participants
- Provide a forum for communication between laboratories to exchange ideas and information.

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