Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Lower serum fibroblast activation protein shows promise in the exclusion of clinically significant liver fibrosis due to non-alcoholic fatty liver disease in diabetes and obesity

K.H. Williams [a,b,c,d], A.J. Viera de Ribeiro [a,e], E. Prakoso [a,b,e], A.S. Veillard [d], N.A. Shackel [a,b,e], Y. Bu [f], B. Brooks [b,g], E. Cavanagh [b], J. Raleigh [b], S.V. McLennan [a,b,c], G.W. McCaughan [a,b,e], W.W. Bachovchin [h], F.M. Keane [a,e], A. Zekry [f,i], S.M. Twigg [a,b,c,1,*], M.D. Gorrell [a,e,1]

[a] Sydney Medical School, The Edward Ford Building (A27), The University of Sydney, NSW, Australia
[b] Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW, Australia
[c] The Charles Perkins Centre, Building D17, Johns Hopkins Drive, The University of Sydney, NSW, Australia
[d] NHMRC Clinical Trials Centre, The University of Sydney, Locked Bag 77, Camperdown, NSW 1450, Australia
[e] Centenary Institute, Locked Bag 6, Newtown, NSW 2042, Australia
[f] Inflammation and Infection Research Centre, School of Medical Sciences, Wallace Wurth Building, University of New South Wales, Sydney, NSW 2052, Australia
[g] Sydney Nursing School, Building M02, The University of Sydney, NSW 2006, Australia
[h] Sackler School of Biomedical Sciences, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA
[i] The St George Hospital, Gray Street, Kogarah, NSW 2217, Australia

ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form
28 January 2015
Accepted 20 February 2015
Available online 11 March 2015

Keywords:
NAFLD fibrosis score
Fibroblast activation protein
Biomarker

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is common in diabetes and obesity but few have clinically significant liver fibrosis. Improved risk-assessment is needed as the commonly used clinical-risk algorithm, the NAFLD fibrosis score (NFS), is often inconclusive.

Aims: To determine whether circulating fibroblast activation protein (cFAP), which is elevated in cirrhosis, has value in excluding significant fibrosis, particularly combined with NFS.

Methods: cFAP was measured in 106 with type 2 diabetes who had transient elastography (Cohort 1) and 146 with morbid obesity who had liver biopsy (Cohort 2).

Results: In Cohort 1, cFAP (per SD) independently associated with median liver stiffness (LSM) ≥10.3 kPa with OR of 2.0 (95% CI 1.2–3.4), $p = 0.006$. There was 0.12 OR (95% CI 0.03–0.61) of LSM ≥ 10.3 kPa for those in the lowest compared with the highest FAP tertile ($p = 0.010$). FAP levels below 730 pmol AMC/min/mL had 95% NPV for LSM ≥ 10.3 kPa and reclassified

* *Corresponding author at*: Sydney Medical School, The University of Sydney, NSW 2006, Australia. Tel.: +61 286271890; fax: +612 8627 1604.
E-mail addresses: kath.williams@sydney.edu.au (K.H. Williams), anajulia.vribeiro@gmail.com (A.J. Viera de Ribeiro), e_prakoso@hotmail.com (E. Prakoso), annesophie.veillard@sydney.edu.au (A.S. Veillard), n.shackel@centenary.usyd.edu.au (N.A. Shackel), koko_bu@hotmail.com (Y. Bu), belinda.brooks@sydney.edu.au (B. Brooks), erikacavanagh@gmail.com (E. Cavanagh), liz.jim.raleigh@bigpond.com (J. Raleigh), sue.mclennan@sydney.edu.au (S.V. McLennan), G.McCaughan@centenary.usyd.edu.au (G.W. McCaughan), william.bachovin@tufts.edu.au (W.W. Bachovchin), f.keane@centenary.org.au (F.M. Keane), a.zekry@unsw.edu.au (A. Zekry), stephen.twigg@sydney.edu.au (S.M. Twigg), m.gorrell@centenary.org.au (M.D. Gorrell).
[1] Equal senior author contributors.

http://dx.doi.org/10.1016/j.diabres.2015.02.024

41% of 64 subjects from NFS 'indeterminate-risk' to 'low-risk'. In Cohort 2, cFAP (per SD), associated with 1.7 fold (95% CI 1.1–2.8) increased odds of significant fibrosis ($F \geq 2$), $p = 0.021$, and low cFAP reclassified 49% of 73 subjects from 'indeterminate-risk' to 'low-risk'.
Conclusions: Lower cFAP, when combined with NFS, may have clinical utility in excluding significant fibrosis in diabetes and obesity.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), including simple steatosis, non-alcoholic steatohepatitis and cirrhosis, is common [1,2]. While the prevalence of NAFLD in the general population is estimated to be up to 40%, it may exceed 90% in some cohorts with both type 2 diabetes and morbid obesity [2–5]. In addition, both type 2 diabetes and obesity are independent predictors for NAFLD progression to clinically significant fibrosis. Significant fibrosis in NAFLD is associated with both liver related and systemic morbidity and mortality and NAFLD is now the third leading indication for liver transplant in the US [1,2,6]. Thus, developing effective and practical non-invasive methods for screening for significant liver fibrosis due to NAFLD in these large, at risk populations with diabetes and obesity is of high clinical importance [2,7].

While liver biopsy is currently the 'gold standard' for grading fibrosis in NAFLD [2,5], this is not a useful method for routine assessment in such large populations at risk. Here, there is a need for readily available, non-invasive assessment of significant fibrosis risk, prior to recommending biopsy [7]. Transient elastography (TE), using ultrasound technology to measure the elastic modulus of liver tissue as a surrogate measure of liver fibrosis, is now being employed in many tertiary centres for non-invasive screening [8]. Algorithms that use simple clinical and biochemical parameters are increasingly recognised as a useful method for stratifying individuals, prior to referral for TE ± liver biopsy [9].

The most externally-validated algorithm to assess for liver fibrosis risk in NAFLD is the NAFLD fibrosis score (NFS) [10]. NFS is based on parameters of: age, body mass index (BMI), diabetes/impaired fasting glucose presence, aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio, albumin and platelets. The calculated NFS is then used to determine whether the patient is low, indeterminate or high risk for fibrosis [10]. The NFS is useful for ruling out clinically significant hepatic fibrosis in NAFLD [11]. Moreover, in several independent series, higher NFS scores have been linked to increased overall, in addition to cardiovascular and liver related, mortality [12–14]. However, limiting its clinical usefulness, up to 60% of individuals are classified as 'indeterminate-risk' for significant fibrosis with uncertain clinical implications [10,11].

The addition of a fibrosis-related biomarker to the assessment of NAFLD may add value to the NFS, particularly to the 'indeterminate-risk' category, without adding significant cost or complexity. As fibroblast activation protein (FAP), also known as antiplasmin-cleaving enzyme, is most similar in structure to dipeptidyl peptidase-4 (DPP4) but with significant expression limited in adults to pathological sites, including in liver fibrosis, it is conceptually a good candidate biomarker [15–23]. DPP4 may be pro-apoptotic and is itself induced by apoptosis within the liver [24]. Hepatocyte apoptosis is thought to be linked to NASH and fibrosis progression and is enhanced by chronic inflammation, oxidative stress and endoplasmic reticulum (ER) stress, all primarily induced by lipotoxicity within the liver [2,25,26]. Uniquely, FAP has both dipeptidyl-peptidase activity similar to DPP4 [16,17], as well as an endopeptidase activity enabling it to cleave many matrix associated proteins such as collagen type-1 and alpha 2-antiplasmin, with overall consequent effects on fibrosis and fibrinolysis [15,16,19,27]. FAP as a trans-membrane, homodimeric protein also exists in an active soluble form that is measurable in the circulation and is elevated in cirrhosis [19,22,23]. While polymorphisms in some genes may associate with and also contribute to liver fibrosis [28–30], genetic variants of the FAP gene appear to be rare [31], and to date, none have been reported that co-segregate with increased FAP gene expression, FAP circulating protein level or cellular function. In contrast, elevated cFAP appears to occur in pathological states such as cirrhosis [19,22,23].

This study sought to explore an association of cFAP with clinically significant liver fibrosis. In particular, it addressed the usefulness of cFAP as a novel biomarker, in combination with the NFS, in the exclusion of significant liver fibrosis secondary to NAFLD in individuals with diabetes and/or obesity.

2. Subjects, materials and methods

Two cohorts were recruited for study:

Cohort 1: From April 2011 until June 2012, individuals aged above 18 years were randomly recruited from a tertiary diabetes centre. All subjects had TE (FibroScan®, EchoSens, France) to screen for severe hepatic fibrosis.
Cohort 2: From July 2006 until April 2010, morbidly obese individuals undergoing bariatric surgery were randomly recruited. In each case, a liver specimen was obtained during surgery by experienced surgeons. Cohort 2 was primarily used to validate results obtained from Cohort 1.

Exclusion criteria for both cohorts were alcohol consumption exceeding 140 g per week and/or 40 g per day, known current hepatitis B or C infection or autoimmune or metabolic liver disease and/or known malignancy or clinically significant heart failure. All individuals underwent a detailed clinical history and examination.