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REVIEW

Red Blood Cell Dysfunction in Non-Alcoholic Fatty Liver Disease: Marker and Mediator of Molecular Mechanisms

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ABSTRACT

Despite efforts to unravel the pathogenetic mechanisms of non-alcoholic fatty liver disease (NAFLD), there is still a need for approved treatments and biomarkers. Interestingly, red blood cells present alterations in their characteristics during NAFLD. The phosphatidylcholine to phosphatidylethanolamine ratio, fatty acid profile, red blood cell count and red cell distribution width reflect molecular changes that are taking place in the liver. In addition, glycosylated hemoglobin, chemokine binding and release, and phosphatidylserine exposure actively participate in NAFLD pathogenesis. In this review, we describe the neglected red blood cell dysfunction in NAFLD, with the aim to unveil potent biomarkers and therapeutic targets.

Keywords: red blood cells, non-alcoholic fatty liver disease, membrane lipids, glycosylated hemoglobin, phosphatidylserine, chemokines.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world, affecting more than 25% of the global population (1). Research in the field has pointed towards a multiple hits hypothesis, where genetic predisposition, epigenetic signature, insulin resistance, lipid mediators, cytokines, hormones, apoptosis, oxidative stress and gut microbiota constitute the main events (2). However, there is still a need for reliable biomarkers for diagnosis, staging, prognosis and monitoring of treatment response (3). Erythrocytes are excellent contributors to the pathogenetic mechanisms of immunometabolic diseases due to their lack of organelles, circulation in the whole body, role in lipid trafficking (4) and evolutionarily conserved immunomodulatory activity (5). In this review, we have tried to describe the

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neglected role of red blood cells as markers and mediators of NAFLD pathogenesis.

The phosphatidylcholine to phosphatidylethanolamine ratio

During NAFLD, the phosphatidylcholine to phosphatidylethanomine ratio (PC/PE) of the erythrocyte membrane is lower than that of healthy subjects. This is accompanied by a concomitant decrease in hepatic PC/PE ratio (6). Similar results have been found in the context of hepatitis C infection, where the PC/PE ratio of erythrocytes was found to constitute a potential therapeutic biomarker (7). The red blood cell PC/PE ratio could provide an important biomarker, representative of the molecular mechanisms taking place in the liver. An important fraction of hepatic PC is synthesized by phosphatidylethanolamine N-methyltransferase (PEMT) (8). Interestingly, decreased levels of PEMT mRNA have been found in patients with NASH, while the same study showed that animals with PEMT deficiency and a high-fat diet developed non-alcoholic steatohepatitis (NASH) (9). In another study, this was associated with a lower PC/PE ratio in the liver, which subsequently reduced plasma membrane integrity (10). In other animal models of steatohepatitis, a low PC/PE ratio in hepatic endoplasmic reticulum was found to be correlated with stress (11). Moreover, a reduced PC/PE ratio has been observed in mitochondria of animals with NASH (12). Thus, we hypothesize that erythrocyte PC/PE ratio in NAFLD could be indicative of the molecular mechanisms associated with lower hepatic PC/PE ratio.

Yet, red blood cell PC/PE ratio could be also affected by systemic inflammation, which results in selective PC hydrolysis in the erythrocyte membrane (13), thus indicating the presence of pro-inflammatory molecules in the blood.

Fatty acid profile

The erythrocyte fatty acid profile was found to strongly reflect the profile of hepatic fatty acids in obese individuals (14). Thus, it is no surprise that the fatty acid profile of red blood cells has been tested in various studies, in the context of NAFLD. Notarnicola *et al* (15) showed that erythrocytes of patients with NAFLD present low levels of stearic acid to oleic acid ratio. In another study, the content of omega-3 poly-unsaturated fatty acids (PUFAs) of the erythrocyte membrane was correlated with fatty liver index (FLI) only in older female patients (16). Contradictory but similarly sex-specific results were reported by Amirkalali *et al* (17). In their study, ω-3 PUFAs were correlated with IL-6 in men and TNF-α in women. Earlier, erythrocyte content in 16:1n7 had been associated with FLI and gamma-glutamyl transferase (GGT) in all patients, and with alanine aminotransferase (ALT) in men. Also, GGT was associated with the content of erythrocytes in 16:1n9 and 18:1n7 in men (18).

The fatty acid profile of erythrocyte membrane has also been examined as a tool for the response to dietary interventions during NAFLD. A seminal study showed that a six-month dietary intervention led to a decrease in the content of palmitic, oleic, palmitoleic and stearic acids, while the levels of PUFAs were increased. This was accompanied by an improvement of the levels of important biomarkers representative of the disease state (19). These studies indicate that the fatty acid profile of red blood cells could provide substantial information regarding the pathogenesis and treatment of NAFLD. The correlation of erythrocyte fatty acids with metabolic and inflammatory markers could be explained by the fact that ω-3 PUFAs comprises important precursors for the biosynthesis of pro-resolving lipid mediators, which participate in the regression of NASH (20–22).

Red cell distribution width

Patients with NAFLD were found to have an increased red cell distribution width (RDW) (23). In addition, other studies have shown that RDW was more elevated in patients with steatohepatitis than those with simple steatosis (24), and even more elevated in NAFLD patients with fibrosis (24, 25). In general, RDW is determined by folate, B12 and iron levels in the body (26). Thus, in the context of NAFLD, RDW could be associated with vitamin B12 and/or folate deficiency (27).

Red blood cell count

Wang *et al* (28) showed a positive correlation between the risk of fatty liver and red blood cell count. The mechanisms indicated by this clinical correlation have not been investigated, but they

could underline the importance of additional anti-oxidant cells during NAFLD.

Glycosylated hemoglobin

Glycosylated hemoglobin (HbA_{1c}) is indicative of long-term glucose levels in the blood. It is a member of the team of molecules termed advanced-glycation end products (AGE), possibly involved in the progression of NAFLD through activation of AGE receptors, which are present in Kupffer cells and hepatic stellate cells (29). The levels of HbA_{1c} are correlated with the existence of NAFLD (30). In fact, the association of HbA_{1c} with the progression of NAFLD remains even in the absence of diabetes (31). Interestingly, HbA_{1c} can predict the development of NAFLD even in individuals with normal levels of HbA_{1c}. In addition, the same study, after bioinformatics analysis, showed that HbA_{1c} could be actively implicated in the pathogenetic mechanisms of NAFLD by activating receptor for advanced-glycation end products (RAGE), inducing hypoxia and suppressing the release of nitric oxide (NO) (32).

Phosphatidylserine exposure

The externalization of phosphatidylserine (PSer) on erythrocyte surface has been found to constitute a male-specific biomarker of metabolic syndrome (33) and high fat-diet (34). This is of major importance in NAFLD, since NAFLD is usually defined as a manifestation of metabolic syndrome in the liver. Otogawa *et al* (35) found erythrocyte accumulation in liver specimens of patients with NASH. In the same study, phosphatidylserine exposure of erythrocytes was observed in animals with steatohepatitis, which led to erythrocyte accumulation in the liver and subsequent erythrophagocytosis by Kupffer cells. This was accompanied by iron accumulation in Kupffer cells and augmentation of inflammation and fibrosis. Hence, PSer exposure in erythrocytes could be a biomarker for disease mechanisms.

CCL2 binding and release

Unruh *et al* (34) showed that high-fat diet triggers increased CCL2 binding by erythrocytes. Our unpublished results indicate that red blood cells from patients with NAFLD also release greater amounts of CCL2. Thus, in combination with increased red blood cell count and increased accumulation in the liver, erythrocytes could contribute to the pool of CCL2 molecules attracting monocytes in the fatty liver (36). ❑

CONCLUSIONS

Red blood cells, due to lack of organelles, do not possess the ability of synthesizing and repairing damaged and modified macromolecules. Thus, the environment through which they circulate can trigger irreversible modifications. These molecular alterations can serve as both biomarkers and mediators of disease. Recent studies have proved that red blood cells are an active player during immunometabolic deregulation. Research upon red blood cell dysfunction in NAFLD could provide novel markers and therapeutic targets. ❑

Conflicts of interests: none declared.

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