Critical review

The role of platelets in the prognosis of renal disease

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Abstract

Introduction

Platelets were first discovered in 1842, identified as 'little globules' or 'granular masses'. Since then, our understanding of these small non-nucleated cells has massively increased, and over the years the humble platelet has been found to be a critical player in many physiological processes. Here, we explore the role of platelets in patients with renal diseases and examine their significance in outcomes.

Conclusion

It is clear that, far from being mere 'particles in the blood', platelets are remarkable and exciting cells that have highly evolved and intricate functions spanning far beyond just haemostasis, encompassing many other physiological processes.

Introduction

Far from the first somewhat tentative identification of platelets as distinct morphologic elements in the blood in the nineteenth century, we have now amassed much information about their structure and function[1]. They are non-nucleated fragments of megakaryocytes. As each megakaryocyte develops in the bone marrow under the control of thrombopoietin, it fragments yielding in excess of 1,000 platelets. In the absence of endothelial activation, platelets circulate in the blood for approximately 10 days before being cleared primarily by the spleen. Despite being simple cytoplasmic fragments, platelets have a remarkable structure, with various surface proteins involved in aggregation and adhesion and secretory granules releasing proteins involved in haemostasis, and the ability to alter their shape and size upon activation.

Platelets are, of course, key players in the process of haemostasis and have long been credited as being fundamental to the formation of stable blood clots upon activation of the coagulation cascade and via their interaction with exposed subendothelial von Willebrand factor (vWF) in the microcirculation. Increasingly, however, they are recognised to have many additional functions and in particular are acknowledged as contributors to vascular inflammation and the development of atherosclerotic disease. They secrete mediators of inflammation (cytokines, chemokines, growth factors, adhesion molecules and coagulation factors) and interact with other cells (dendritic cells, leukocytes and progenitor cells) to promote their activation and recruitment to sites of inflammation[2]. The aim of this review is to discuss the role of platelets in the prognosis of renal disease.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Inflammation and cardiovascular disease

Before examining the role of platelets in inflammation, it is important to remember that it has been long known that inflammation is involved in the development of atherosclerosis in the general population[3,4]. Atherosclerosis is now very much recognised as being a chronic inflammatory condition of the vessel wall, with the development of atherosclerotic lesions being shaped by immune responses and regulations[5] and the haemostatic system acting as a moderator[6]. Elevated levels of hs-CRP and plasma proinflammatory cytokines, in particular the IL-1 and IL-6 pathways, have been repeatedly implicated in atherogenesis[7].

Equally, patients with chronic kidney disease (CKD) are well recognised to be in a proinflammatory state[8] with evidence that CRP, IL1, 6 and TNFa are elevated alongside ICAM-1 and VCAM-1 and act as predictors of cardiovascular death[9].

Platelets in inflammation

Platelets have been identified as being effector cells that enhance inflammatory responses, with the ability to 'cross-talk' with endothelial cells and leukocytes[10]. When activated, they release over 300 proteins and molecules—some (including chemokines, angiogenic factors, ADP/ATP, coagulation factors) preformed and stored in dense bodies or alpha-granules, others (thromboxane, reactive oxygen species, IL-1b) synthesised upon stimulation[11].

In addition to secreting these various biologically active factors, activated platelets have also been shown to shed membrane microparticles (MPs)[12]. MPs are phospholipid- and protein-rich submicron particles 0.1–2 mm in size. They shed from the membranes of several cell types when they are injured, activated or undergoing apoptosis in response to

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cytokines, thrombin, endotoxin, hypoxia and shear stress[13]. Cells known to shed MPs include platelets and endothelial cells. The most abundant MPs in the circulation are those from activated platelets, and they are thought to generate and deposit various complement components[14]. MPs are believed to be involved in atherogenesis, being found in higher levels in those with severe hypertension and those with increased coronary heart disease risk[15]. They have been implicated as a prognostic marker for atherosclerotic vascular disease[12]. In addition, endothelial MPs are involved in inflammation and vascular function.

Another key factor linking platelets with inflammation may take the form of microRNAs. MicroRNAs are small non-coding RNA molecules that function in transcriptional and post-transcriptional regulation of gene expression. Those that regulate immune cell differentiation would thus be expected to be important during plaque formation. It has been shown that microRNAs contribute to megakaryocytopoiesis, are expressed in platelets and are involved in defining platelet function[5].

An additional point of interest is one of the proinflammatory mediators released from platelets, myeloid-related protein 8/14. This has been investigated as a biomarker for adverse cardiovascular events. Interestingly, MRP 8/14 can also activate innate immunity via interaction with TLR-4 as well as promoting endothelial cell apoptosis, further serving to illustrate the link between inflammation and thrombosis[16].

Interest has also been given to platelet size, which has been found to be linked with platelet activity. The mean platelet volume has been found to be associated with cardiovascular risk and, due to the relative ease of measuring it (available with routine blood counts), has been proposed as a potential tool for identifying high-risk patients[17].

As well as platelet size, the ratio of platelets to other circulating cells has been used as tool for identifying inflamed and subsequently high-risk patients. Neutrophil-to-lymphocyte ratio has previously been suggested as a potential marker to determine inflammation in CKD patients. Recently, platelet–lymphocyte ratio has also been investigated. It is known that platelet–lymphocyte ratio is positively correlated with inflammatory markers such as TNFa and IL6 in cardiac patients[18], and this has now also been shown to predict inflammation, and thus potentially cardiovascular risk, in the CKD population[19].

Effect of uraemia on platelets
Since the advent of dialysis, it has been clear that uraemia has an effect on platelet function, as evidenced by the key clinical problem of excessive bleeding. It has been identified that there are several contributing factors to platelet dysfunction in uraemia—various platelet abnormalities, abnormal platelet–vessel wall interaction and altered interaction with other circulating cells included. Given the excess of cardiovascular morbidity and mortality in patients with CKD, the evidence that atherosclerosis is an inflammatory condition and the fact that dialysis patients are known to be chronically inflamed, it would seem plausible that the abnormalities in platelets seen in this population would also play a role in their excessive cardiovascular risk.

Platelet function in uraemia
The most common abnormality is prolongation of bleeding time, with multiple platelet abnormalities contributing to defective aggregation and a delay in time to formation of the primary haemostatic plug[20]. Among the platelet abnormalities seen in uraemia are listed abnormal granule content and release, abnormal arachidonic metabolism, abnormal cyclo-oxygenase activity, abnormal handling of cAMP, intracellular calcium,

serotonin and abnormal binding of GP IIb-IIIa[21]. Furthermore, uraemic platelets have attenuated response to thrombin, with reduced secretion of ATP and other granule contents[22], and have diminished ATP release to arachidonic acid, producing an aspirin-like effect as evidenced by platelet aggregation studies[23]. Also interestingly, the abnormalities seen in intracellular calcium may be linked with ambient parathyroid hormone concentration, and thus hyperparathyroidism may further affect platelet reactivity[24].

It has also been shown that, in addition to these baseline abnormalities, platelet reactivity and aggregation are altered throughout the course of a dialysis session, with similar increases in reactivity as seen in patients with coronary artery disease[25].

Interaction with vessel wall
The fact that platelets do not adhere to normal endothelial cells has been ascribed to inhibition by nitric oxide (NO), prostacyclin and adenosine, which are known to be generated by healthy vascular endothelium. Interestingly, it has been shown that uraemic platelets placed in normal plasma retain normal function, thus suggesting a putative uraemic toxin or toxins[21]. The role of NO forms an interesting link. NO limits both platelet–platelet interaction and also platelet adhesion to endothelium by modulating vascular tone. In normal subjects, it prolongs the bleeding time. We have shown that platelets from haemodialysis patients generate more NO than healthy subjects[26]. Furthermore, guanidinosuccinic acid (GSA) has also been shown to significantly lengthen bleeding time, and this effect is attenuated by a specific NO inhibitor, with evidence suggesting that NO formation by uraemic vessels is GSA dependent. GSA accumulates in uraemia as part of an alternative pathway for ammonia detoxification[21].

Furthermore, platelet interaction with the endothelium may be disrupted by abnormal interaction with vWF. It has been demonstrated that glycoprotein expression is lower on resting platelets in patients with CKD, and that this reduction correlates with the severity of renal impairment. The same investigators also found that GP1b expression on stimulated platelets increases in haemodialysis patients. GP1b is involved in platelet adhesion to the endothelium and is the vWF receptor[27].

There is also an interesting association with anaemia. Bleeding time is further prolonged in uraemia patients who are also anaemic, proportionately to the degree to anaemia. This is ameliorated when the haematocrit is increased to at least 30%[28]. The correction occurs as more erythrocytes in the circulation push more platelets and leukocytes to the periphery, allowing improved contact with the endothelium and improved stability of the primary haemostatic plug[20].

Interaction with other circulating cells
As part of their role in inflammation, platelets aggregate with leucocytes via their P-selectin receptor interacting with its natural ligand P-selectin glycoprotein ligand-1 on monocytes and neutrophils. P-selectin is translocated to the surface of activated platelets where it contributes to platelet-assisted enhancement of thrombosis at sites of endothelial injury[11,29].

These aggregates form an anchoring source for inflammatory cells on activated platelets and contribute to ongoing injury at the sites of atheromatous plaques. Levels of platelet–monocyte (PM) aggregates have been found to be significantly higher in dialysis patients. In patients with normal renal function, PM aggregates have been associated with cardiovascular disease. Data from our unit would suggest that this also applies in uraemic patients, with a significant increase in cardiovascular morbidity and mortality seen in those patients with higher levels of circulating PM aggregates[29].

Effect of platelets in uraemic patients
In light of the various platelet aberrancies discussed above in uraemic patients, it would follow that platelets are crucial players in the prognosis of patients with CKD. Both thrombocytosis and thrombocytopenia are commonly seen in patients with end-stage renal disease (ESRD), and the role of antiplatelet agents in these patients is, at best, controversial.

Thrombocytosis
Relative thrombocytosis has been linked with severity of cardiovascular disease in the CKD population. In peritoneal dialysis patients, it has been demonstrated that relative thrombocytosis (platelet count >300) correlates significantly with both coronary and peripheral artery disease[30].

In long-term haemodialysis patients, it has also been shown that a platelet count of >300 is associated with an increased death rate[31]. The investigators here linked this relative thrombocytosis with a reduction in iron stores, which may also be a key player.

Another study published in 2011 suggested that relative thrombocytosis in CKD patients was associated with higher all-cause and cardiovascular death, by means of its association with malnutrition inflammation cachexia syndrome (MICS). They found that patients with a platelet count >300 had poorer cardiovascular outcomes, with mortality rates higher the greater the platelet count. Interestingly, when they adjusted for MICS, these associations were negated, suggesting that relative thrombocytosis is a marker of a worse MICS profile, thus accounting for their increased morbidity and mortality[32].

Thrombocytopenia
Conversely, patients with thrombocytopenia and CKD are also commonly seen. A reduction in platelet count during the course of a dialysis session is recognised, alongside platelet activation and degranulation, which is attributed to exposure to the dialysis membrane and the roller pump and results in platelet–lymphocyte aggregates[29,33]. Thrombocytopenia may also be seen as part of the syndrome of heparin-induced thrombocytopenia in dialysis patients, whereby the generation of the platelet-factor 4/heparin complex triggers antibody formation. The complex then binds to the antibodies, cross-reacts with platelet surface receptor activation and aggregation, further PF4 release and formation of procoagulant factors and thrombin. It has been suggested that the presence of these antibodies is an independent predictor of cardiovascular morbidity and mortality[34,35].

Rejection
Humoural rejection can be seen as an extension of platelet–endothelial dysfunction. Indeed, it has been shown that, after primary injury to endothelium, platelet aggregation is seen as the earliest morphological change and may precipitate release of platelet granules stimulating the endothelial activation and vascular damage that is seen in rejection[36]. Indeed, over two decades ago, the use of indium-111 platelet scintigraphy was examined in the detection of renal transplant rejection when it was suggested that platelet deposition was a potential tool for early detection of acute graft rejection[37].

Experimentally, it has been suggested that platelet aggregation is seen within a few minutes of reperfusion of a newly transplanted organ and that these activated platelets may contribute to the extent of the injury seen in the graft. It has also been demonstrated that activated platelets can cause inflammation

and ischaemia of previously normal endothelium[36]. Is it reasonable to suppose, therefore, that activated platelets at the time of transplantation may go on to cause endothelial injury.

HUS/TTP

The link with platelets and endothelial injury in renal patients is also seen clearly in the thrombotic microangiopathies, whereby abnormal platelet aggregation and thrombotic occlusion of the microvasculature is responsible for the clinical syndromes seen. The role of vWF and the ability of ultralarge vWF to enhance platelet aggregation and adhesion to the subendothelium has been extensively examined in these conditions[38]. These interactions are widely reported and beyond the remit of this review, but adds just another example of the importance of platelets in renal disease.

The use of antiplatelet agents in uraemia

Given the plethora of platelet abnormalities and bleeding complications seen in uraemic patients, it would seem obvious that antiplatelet agents should be avoided at all costs. But the fact remains that ESRD patients have a significantly elevated cardiovascular disease burden, thus suggesting that they may play a role in primary prevention. A comprehensive review of the literature is out of the scope of this article, but suffice to say that there is an increasing body of research regarding the use of antiplatelets and oral anticoagulants in the CKD population[39]. A recent meta-analysis suggested that antiplatelet agents do reduce myocardial infarction in such patients but with significant increases in major bleeding, which may outweigh any potential benefits[40]. Data published on major bleeding events in dialysis patients gives a frequency of events of 2.5% per person-year, increasing to 4.4% with the use of aspirin alone and

6.6% with its concomitant use with warfarin[41]. These data certainly add weight to the fact that any prescription of anti-platelet agents in CKD patients should be considered on individual risk and benefit ratios.

Conclusion

It is clear that, far from being mere 'particles in the blood', platelets are remarkable and exciting cells that have highly evolved and intricate functions spanning far beyond just haemostasis, encompassing many other physiological processes. Their link with CKD and its associated complications is beginning to be unravelled. Platelets are a worthy topic of further study as we try to resolve the conundrum of inflammation and its deleterious, cardiovascular and other effects in such patients.

Abbreviations list

CKD, chronic kidney disease; ESRD, end-stage renal disease; GSA, guanidinosuccinic acid; MICS, malnutrition inflammation cachexia syndrome; MP, microparticle; NO, nitric oxide; PM, platelet–monocyte

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