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Platelets in Alzheimer's Disease

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that gradually leads to severe cognitive impairment. The neuropathological hallmarks of AD include beta-amyloid plaques, tau neurofibrillary tangles, inflammation and glial responses, vascular dysfunction, synapse loss and cholinergic neurodegeneration. Currently, the diagnosis of possible or probable AD is based on a time consuming combination of clinical and psychological testing, imaging, and the analysis of three well-established biomarkers (beta-amyloid(42), total tau and phospho-tau-181) in cerebrospinal fluid. The search for biomarkers in blood is of high importance to avoid invasive lumbar puncture and to allow fast and easy analysis of a high number of patients. Biomarkers have been screened in blood of AD patients in plasma/serum, peripheral blood mononuclear cells (PBMCs), monocytes or also in platelets.

Platelets are interesting targets to study AD because they share some properties with neurons: they contain the neurotransmitter serotonin and the amyloid-precursor protein (APP), which produces the beta-amyloid, which aggregates in the brain of AD patients. Moreover, platelets are an easily accessible source of human cells. This review focuses on changes in the platelets of AD patients and will summarize (1) platelet activation including mean platelet volume, membrane fluidity and coated platelets, (2) serotonin metabolism, (3) APP isoforms and processing enzymes, including secretases, (4) oxidative stress and radicals, including nitric oxide metabolism and mitochondrial pathologies, including cytochrome-c oxidase and monoamino-oxidase-B and (5) enzymatic activity, such as glycogen synthase kinase-3 or phospholipase A2. In spite of all efforts, the discrepant results so far have prevented the establishment of a valid platelet-derived AD biomarker. With this survey we provide a detailed review about the major current findings on the potential use of platelet markers in AD.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by progressive deterioration in cognition leading to premature death. The neuropathological hallmarks of AD include beta-amyloid (A β) plaques, neurofibrillary tau tangles, inflammation

and glial responses, synapse loss and cholinergic neurodegeneration among others. The worldwide prevalence of dementia was estimated as high as 24.2 million by 2005 and is expected to quadruplicate by 2050 [1, 2]. North America and Western Europe show the highest prevalence rate of dementia for persons >60 years [3]. Most dementia cases (70%) are attributed to AD, placing a considerable burden on society [2]. The increasing rate of AD cases not only stresses the establishment of efficient therapeutic intervention but also the identification of economic and reliable biomarkers. In both domains great efforts and smaller progress have been made in the last decades, while the desired breakthrough has yet to be awaited.

AD is classified into clinically indistinguishable early onset genetic AD (onset < 60 years) and late onset sporadic AD (> 60 years). Early onset AD accounts for approx. 1-5% of all cases and is associated with a more rapid progression of the neurodegenerative process and autosomal dominantly inherited mutations with a high penetrance. Sporadic AD accounts for >95% of all dementia cases and turned out to be a more heterogeneous disease. Currently, the diagnosis of possible or probable AD according to the NINCDS-ADRDA criteria is based on a time consuming combination of psychological testing, imaging, and the analysis of three well-established biomarkers (beta-amyloid(42), total tau and phospho-tau-181) in cerebrospinal fluid (CSF).

Biochemical markers mirror the physiological changes and can be objectively measured and evaluated as an indicator of pathological changes. A good biomarker must be sufficiently sensitive to detect early changes and specific to differentiate AD from clinically similar conditions. Besides high diagnostic and prognostic accuracy, biomarkers should allow global reproducibility with non-invasive and easy-to-perform tests. Within the search for peripheral AD-specific biomarkers, anucleated blood platelets have shown to be a promising target, since they represent the principal component of human blood affected by (early) biochemical alterations during AD. Moreover, platelets are an easily accessible source of human tissue and contain proteins found in neuronal cells.

In this chapter we aim to review changes in AD occurring in platelets to understand whether they may have a potential as putative biomarkers for diagnosing AD. We will focus on (1) platelet activation including mean platelet volume, membrane fluidity and coated platelets, (2) serotonin metabolism, (3) APP isoforms and processing enzymes, including secretases, (4) oxidative stress and radicals, including nitric oxide metabolism and mitochondrial pathologies, including cytochrome-c oxidase and monoamino-oxidase-B activity and (5) enzymatic activity, such as glycogen synthase kinase-3 or phospholipase A2. In spite of all efforts made, the discrepant results so far have prevented the establishment of a valid platelet-derived AD biomarker. With this survey we provide a detailed review about the major current findings on the potential use of platelet markers in AD.

2. Platelet activation

Vascular risk factors were traditionally considered to distinguish between vascular dementia and AD. However, in the last decade a series of studies revealed that vascular events are

involved in the development of AD and the arbitrary classification into vascular dementia and AD is very much outdated [4-7]. Several studies reported vascular alterations such as an increased number of fragmented vessels, atrophic string vessels, changed vessel diameters, altered capillary membrane and collagen accumulation in the basement membrane of AD patients [8, 9]. Besides, deposits of A β in cerebral vessels (cerebral amyloid angiopathy (CAA)) induce severe damage of the vessel wall and alter the cerebral blood flow promoting the progression of AD [10]. Moreover, it became clear that AD associated alterations are not solely limited to the brain and occur in vessels and blood cells of the peripheral system [11].

Activation and aggregation of platelets are important steps for haemostasis at sites of vascular injury, while uncontrolled activation can trigger thrombotic vessel occlusion at sides of atherosclerotic plaque rupture [12] and lead to chronic inflammatory reactions. In AD, activated platelets are strongly linked to vascular processes and are proposed to be the missing link for the association between atherosclerotic events and AD [13]. Adhesion and activation of platelets on the vascular wall progressively lead to vascular inflammation and atherosclerosis, thus playing a key role in the development of AD-associated conditions. Increased platelet activation has been identified in the late 90s, due to damaged cerebral endothelial cells or membrane abnormalities in the AD brain [14].

Moreover, increased platelet activation, measured by GPIIb-IIIa complex activation or P-selectin expression is significantly higher in AD patients with fast cognitive decline compared to slow cognitive decline [13]. Furthermore it is known, that peripheral A β peptides contribute to platelet adhesion and activation in the initiation of thrombus formation [15, 16]. Recently it has been shown that activated platelets aggregate at sites of vascular A β promoting CAA by inducing platelet thrombus formation leading to vessel occlusion at vascular A β plaques [17, 18]. Because platelets are major players in blood flow alterations and vascular diseases, it was suggested that they do not only mirror AD related changes, but promote actively the progression of AD. In light of these findings it is conceivable that increased platelet activity could induce the progression of AD by contributing to peripheral vascular damage and endothelial senescence. Uncontrolled activation of platelets in AD-subjects may result in chronic inflammation mediating endothelial cell stress, which, in turn, may trigger platelet activation [19]. Alternatively, systemic inflammation in AD patients may result in platelet activation creating a vicious, self-amplifying circle [20]. Thus, it is conceivable that activated platelets contribute at different sites to the progression of AD.

References	Effect on platelet activation
Sevush et al., 1998	↑ in AD
Stellos et al., 2010, 2014	↑ in AD with faster cognitive decline compared to AD with slow decline ↑ in coronary artery disease with cognitive impairment

Table 1. Platelet activation in AD

2.1. Effects on platelet volume

The platelet volume is a marker of platelet activation and thus involved in the pathophysiology of multiple pro-inflammatory diseases. Along with platelet activation, platelet volume values are considered indicators for vascular events linked to cerebral vascular dementia. In AD, both increased [21, 22] and decreased [23, 24] mean platelet volume has been reported. These heterogeneous results point out the importance of establishing methodological consensus in the isolation and processing of platelets, since they are very sensitive to cellular damage.

References	Effect on platelet volume
Yagi et al., 1984	↑ in vascular dementia compared to AD
Yesil et al., 2012	↑ in AD
Wang et al., 2013	↓ in AD and MCI; associated with MMSE
Liang et al., 2013	↓ in vascular dementia and AD

Table 2. Platelet volume in AD

2.2. Effects on membrane fluidity

Discrepant results have been published concerning platelet membrane fluidity: while decreased fluidity of cell membranes from platelets is associated with normal ageing, increased internal membrane fluidity is one of the first alterations to be reported in platelets [25, 26]. Increased platelet membrane fluidity becomes apparent by a decrease in the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) in labelled membranes. It was proposed, that this apparent abnormality of membranes in AD patients is due to an increase in internal membranes [26]. However, no significant differences in either cholesterol or phospholipid compared to healthy subjects has been observed [26], while an increase of intracellular platelet membranes in AD patients has been reported [27]. Other findings suggest furthermore that demented patients with increased platelet membrane fluidity display an earlier onset and a more rapid deterioration of cognitive symptoms compared to other demented subjects [25]. In light of these findings, platelet membrane fluidity was proposed as a biological risk factor for AD, since it is associated with significant contributions to the risk of developing AD [28, 29]. However, others failed to demonstrate differences in platelet membrane fluidity between AD patients and healthy controls, concluding that platelet membrane fluidity cannot be considered as antemortem biomarker for AD [30-32]. An increase in DPH fluorescence anisotropy has been reported [33-36] and as anisotropy is inversely related to membrane fluidity, these results indicate in contrast to the previously mentioned studies a decrease of the external and internal membrane fluidity in AD [33]. It was suggested that the short life span of platelets makes them less susceptible for long-term modifications. And finally, a reduced fluidity in the platelet inner mitochondrial membrane has been reported, which was associated with oxidative damage [37], while others correlated membrane fluidity with alterations of APP fragments [38].

References	Effects on membrane fluidity
Zubenko et al., 1987, 1990	↑ in familial AD by fluorescence anisotropy
Kozubski et al., 1999, 2002	↑ in AD with 2 spin-labelled markers
Cohen et al., 1987; Piletz et al., 1991	↑ by fluorescence anisotropy, ↓ ratio of cholesterol to phospholipid
Kaakkola et al., 1991	↑ in AD and multi-infarct dementia compared to controls
Ortiz et al., 2008	↓ fluidity of platelet inner mitochondrial membrane
Fernandes et al., 1999	unchanged in AD by fluorescence anisotropy
Kukull et al., 1992	unchanged
Kálmán et al., 1994	↑ in AD by fluorescence anisotropy
Cardoso et al., 2004	unchanged in AD compared to controls
Zainaghi et al., 2007	membrane fluidity correlated with alteration of APP fragments
Vignini et al., 2013	↓ in AD by fluorescence anisotropy, worse profile for male patients
Hajimohammadreza et al., 1990	↑ in AD by fluorescence anisotropy

Table 3. Platelet membrane fluidity in AD

2.3. Coated platelets

Coated-platelets (PLTs) are a subset of platelets produced upon dual-agonist stimulation with collagen and thrombin retaining several procoagulant α -granule proteins on their surface [39]. PLTs are important for the coagulation cascade because of their ability to generate thrombin at sites of vascular damage. Effectively, it was suggested that increased levels of PLTs may be related to prothrombotic conditions [40], while decreased levels of PLTs were linked to an increased risk for haemorrhage [41]. It was also demonstrated that coated-platelet levels are elevated in amnesic mild cognitive impairment (MCI) and correlate with the progression of AD [40, 42-45]. Additionally, the same group showed that MCIs with elevated coated-platelet levels are more likely to develop AD, while they found no significant alterations in patients with frontotemporal lobe dementia [44, 45]. So far, coated platelets seem to be an interesting target, which needs to be reproduced internationally by other research groups.

3. Serotonin

Several studies report abnormalities in serotonin (5-HT) concentration as well as alterations in its uptake during AD, possibly linked to psychobehavioral problems such as e.g. depression [46-48]. Platelets accumulate high levels of serotonin in dense granules and release it upon activation [49]. Early studies failed to demonstrate altered serotonin processing in platelets [50,

51], while in the early 90s heterogeneous results regarding 5-HT Km (Michaelis-Menten constant) and maximal velocity (Vmax) binding affinity were published [52, 53, 54]. Compared to controls, patients with AD demonstrated in different studies significantly lower platelet serotonin concentrations [55-58]. However, an increased 5-HT concentration in low-density platelet populations was recently reported [59]. Likewise, heterogeneous results have been reported for serotonin uptake: both increased and decreased serotonin uptake was found in AD platelets [52, 60]. In light of these data and despite great efforts, so far platelet-derived serotonin has not been established as a reliable biomarker for AD.

References	Effects on serotonin
Tukiainen et al., 1981	unchanged 5-HT uptake in
Andersson et al., 1991	unchanged maximum number of binding sites (Bmax) and binding affinity
Inestrosa et al., 1993	↓ ability to accumulate 5-HT ↓ Km and Vmax
Kumar et al., 1995a	↓ 5-HT concentration
Kumar et al., 1995b	↑ affinity of binding of 5-HT to the platelet membrane in AD (only females tested)
Arora et al., 1991	↑ Vmax 5-HT-uptake in mild and moderate AD Trend ↓ Vmax 5-HT-uptake in severe AD
Koren et al., 1993	↓ [3H]-5-HT uptake
Mimica et al., 2008	↓ 5-HT concentrations in non-psychotic female and psychotic male AD patients compared to controls
Muck-Seler et al., 2009	↓ 5-HT concentrations in the late phase of AD compared to other phases and controls
Milovanovic et al., 2014	↑ in low-density platelet populations
Prokselj et al., 2014	↓ 5-HT concentrations in AD compared to AD controls
Vmax = maximum number of 5-HT uptake sites; Km = Michaelis-Menten constant; EOAD = Early Onset AD	

Table 4. Platelet 5-HT in AD

4. Amyloid Precursor protein (APP) and secretases

4.1. APP isoforms and ratios

APP is an integral membrane protein with a large extracellular domain and a shorter, intra-cellular C-terminal tail. Three major APP isoforms (770, 751 and 695 kDa) have been described.

APP 751 und APP 770 contain a Kunitz-type serine protease inhibitor domain (APP KPI), while APP 695 lacks this domain. The APP isoforms are cut by different enzymes (secretases) into smaller peptides, whereas sequential cleavage by β -secretase (BACE1) and γ -secretase (ADAM-10) generates the neurotoxic A β fragments. Conversely, cleavage by α -secretase precludes the formation of amyloid fragments by processing APP within the A β domain.

Platelets are of particular interest in AD research, because they contain high levels of APP [61-63]. In contrast to neuronal tissues where isoform 695 lacking the KPI domain is the most abundant one, platelets express mainly APP770 whereas APP695 is marginally present [64]. Platelets contain α -, β -, γ - secretase activities and generate different APP fragments: sAPP α , sAPP β , the amyloidogenic fragment (C99) and A β peptides [65, 66]. Platelet APP is mainly processes by the α -secretase pathway releasing soluble APP (sAPP) [66] and predominantly A β (40). Both APP and A β are stored in α -granules of platelets and become released upon activation by agents like the physiological agonists thrombin and collagen. A recent study reports significant up-regulation of platelet APP isoforms compared to controls, and a correlation between APP mRNA levels and cognitive impairment [67]. The same group found significant up-regulation of platelet mRNA expression level of total APP and APP containing a KPI domain in patients with AD and frontotemporal lobe dementia compared to controls [68]. We have recently shown that platelet-secreted APP β in MCI and AD is significantly increased when measured with ELISA compared to control subjects, while no changes in sAPP α are seen [69].

References	APP expression and release
Vignini et al., 2013	\uparrow mRNA of total APP and APP containing KPI domain in AD and FTLD \uparrow APP isoforms compared to controls
Marksteiner and Humpel, 2013	\uparrow sAPP- β in MCI and AD compared to controls unchanged sAPP- α in MCI and AD compared to controls

Table 5. Platelet APP expression in AD

Several studies showed that the platelet APP ratio (defined as the ratio between the upper 130kDa and the lower 106-110kDa isoforms) is significantly lower in AD patients compared to controls and patients with other forms of dementia [70-77]. It seems that the alteration of platelet APP isoforms is an early event in AD and the ratio shows to be a consistent predictor for the conversion from MCI to AD. In fact, MCI subjects converting to AD showed significantly decreased APP ratios at baseline compared to other dementia forms and stable MCI subjects [78-80]. Furthermore, the APP ratio positively correlates with cognitive decline, i.e. the lower the ratio, the more severe the disease [38, 73]. Furthermore, it has been shown that carriers of the APOE4 allele are associated with a larger reduction in the APP ratio [81]. Moreover, administration of acetylcholine esterase inhibitors [82, 83] increases the ratio of APP forms in AD suggesting a possible effect of these drugs on APP trafficking in platelets. The

proposed cut-off scores are around 0.56 with a sensitivity of 88% and specificity of 89% [80, 84]. Thus, the APP ratio has been proposed as a potential biomarker in prodromal AD-stages and a reliable indicator for the disease progress. Despite the promising homogeneous findings the validity of APP ratio as a useful supportive biomarker for AD diagnosis is not yet internationally established. Methodological problems including lack of sensitivity and diversity of the used antibodies and different isolation procedures of platelets may account for this problem.

References	Effects on APP ratio
Di Luca et al., 1996, 1998	↓ in AD and elderly patients with Down Syndrom; ratio correlated with the severity of the disease
Rosenberg et al., 1997	↓ in AD compared to controls
Baskin et al., 2000, 2001	↓ specific in AD compared to PD and HS (Hemorrhagic Stroke); unchanged in cognitively normal young adults carrying PS-1 mutations
Padovani et al., 2001, 2002	↓ in MCI and mild and very mild AD
Borroni et al., 2002, 2003, 2004	↓ in MCIs converting to AD and early stages of probable AD
Liu et al., 2005	↓ in AD; increases with galantamine treatment for 12 weeks
Tang et al., 2006	↓ in AD
Zainaghi et al., 2007, 2012	↓ in MCI converting to dementia upon follow-up
Srisawat et al., 2013	↓ in Thai patients with AD

Table 6. Platelet APP ratio in AD

4.2. Enzymatic activity: BACE1 and ADAM-10

APP is cleaved into secreted (soluble) APP (sAPP), smaller intracellular fragments and the Aβ peptides (40, 42 or 43 amino acids) by three secretases (α, β, γ). The α-secretase leads to the non-amyloidogenic pathway, while cleavage of APP by β -secretase (BACE1) and γ-secretase (ADAM-10) generates the toxic Aβ fragments. So far, several authors report increased BACE1 in AD by Western blotting or ELISA analysis [69, 73, 85, 86]. However, using a novel ELISA system a significant decrease of BACE1 N-terminal and C-terminal fragments has been reported in AD [87]. ADAM-10 is the major constitutive α-secretase for APP processing [88, 89]. To date, platelet ADAM10 (a disintegrin and metalloprotease) has been reported to be significantly reduced in platelets in AD [85, 90, 91] while others failed to detect changes in alpha- and beta-secretase activities in AD [92]. Moreover, reduction in ADAM-10 levels correlates with the progression and seems to be stage-dependent [91].

References	Effects on secretases
Johnston et al., 2008	↑ platelet membrane β -secretase activity in AD compared to controls;
Colciaghi et al., 2002, 2004	↓ ADAM10 in AD compared to controls ↓ level of alpha APPs ↑ BACE1 in AD
Tang et al., 2006	↑ BACE1 in AD ↓ ADAM10 in AD
Gorham et al., 2010	unchanged alpha- and beta-secretase activities and -ratio in AD and MCI
Decourt et al., 2013	↓ BACE1 in AD (newly developed ELISA)
Manzine et al., 2013	↓ ADAM10 in AD

Table 7. Platelet secretase activity in AD

4.3. Activation of platelets by beta-amyloid

It is well known that different heterogeneous amyloidogenic peptides ($A\beta(1-40)$, $A\beta(1-42)$, $A\beta(25-36)$) as well as aggregated $A\beta$ can induce platelet aggregation [15, 16]. Platelets store and release preferentially the 40 amino acid $A\beta$ fragment in their granules upon stimulation with physiological agonists like thrombin, collagen or calcium ionophores [93, 61, 94, 95]. Once released, $A\beta$ peptides trigger platelet activation, initiating a vicious feedback loop of platelet activation and $A\beta$ release. Further, apoptotic stimuli significantly increase platelet $A\beta(40)$ but not $A\beta(42)$ suggesting that this pathway determines altered APP processing [96, 97]. Recently it was shown that $A\beta$ induces platelet activation independent of known physiological agonists [16]. It was furthermore suggested that platelets modulate aggregation of soluble $A\beta$ into fibrillar $A\beta$ and facilitate platelet adhesion at vascular $A\beta$ accumulations, contributing to the full occlusion of the affected vessel [18]. At this point it seems therefore likely that platelets and platelet-derived $A\beta$ may contribute to a significant degree to the amyloid burden in the vascular walls promoting CAA in AD patients [96].

5. Oxidative stress, radicals and mitochondrial pathologies

Excessive chronic oxidative stress and production of radicals in the AD brain has been considered to promote cellular degeneration. Especially, nitric oxide (NO) and peroxynitrite (ONOO) are very reactive toxic radicals (ROS). It becomes more and more clear that a dysregulation of mitochondria and the involvement of cytochrome C-oxidase may play a role in this process. Vascular damage and endothelial dysfunction may play a role in AD and platelets serve as a source of oxidative stress. Mitochondria are the major sites responsible for more than 90 % of the ROS generation. In AD, mitochondrial DNA of cortical neurons was reported to induce excessive oxidative damage and increased DNA mutations [98-100]. Moreover,

abnormal mitochondrial size and decreased mitochondrial number in AD and MCI are likely to increase ROS generation and oxidative damage [99, 101]. In platelets reduced Complex IV and Complex III activity has been repeatedly associated with AD [102-105, 32]. Subsequently, A β was shown to interact directly with mitochondria and inhibit platelet Complex IV activity inducing oxidative stress [106].

5.1. Nitric oxide and peroxynitrite

An increase in nitric oxide synthase (NOS) has been associated with normal aging and with AD [107, 108, 33]. Similarly to NO, peroxynitrite (ONOO) was found to be increased in AD patients and both NO and ONOO are linked to reduced Na⁺/K⁺-ATPase activity in platelet membranes of AD patients [108]. Additionally, carriers of the epsilon 4 allele of apolipoprotein E (APOE) show higher NOS compared to non-carriers [109]. In contrast, others found significantly lower NO concentrations in platelets of AD patients and a generally higher platelet aggregation rate. Since NO is known to inhibit platelet aggregation, NO might be responsible for the aggregation of platelets in the observed AD cohort [110].

References	Effects on nitric oxide
Kawamoto et al., 2005	↑ NO and peroxynitrite in AD
Vignini et al., 2007, 2013;	↑ NO and ONOO(-) peroxynitrite in AD
Marcourakis et al., 2008	↑ NOS activity in APOE epsilon 4 carriers
Yu and Jia, 2009	↓ NO and eNOS (endothelial nitric oxide synthase)

Table 8. Platelet nitric oxide and peroxynitrite concentrations in AD

5.2. Cytochrome c oxidase

Cytochrome C oxidase (COX) is an enzyme located in mitochondria and may play a role in the production of radicals. In most studies, reduced platelet COX activity has been found in AD [111-113, 102, 103, 32, 104] but also in cognitively normal individuals with a maternal history of AD [114]. However, another study did not find any differences in COX activity in AD [115]. Recently, reduced mitochondrial COX activity has been found, which correlates to decreased mitochondrial membrane potential, resulting in higher lipid peroxides, superoxide radicals and protein carbonyls [111]. It has been proposed, that reduced COX activity causes higher tissue vulnerability and reduced oxygen availability [104].

5.3. Monoamino-oxidase B

Monoamino-oxidase-B (MAO-B) is an important enzyme located in the mitochondria and plays a role in metabolic processes of serotonin. Studies on platelet MAO-B activity yielded inconsistent results up to date: increased MAO-B activity was reported by several groups [116-126], while few researchers could not find any abnormalities [127,128]. The decline of mini-mental state examination scores (MMSE) preceded the elevation of MAO-B activity

References	Effects on COX activity
Silva et al., 2013	↓ COX, mitochondrial membrane potential , cytochrome c content
Burbaeva et al., 2012	↓ COX in AD and MCI
Mosconi et al., 2011	↓ COX activity in cognitively normal individuals with a history of maternal LOAD
Cardoso et al., 2004	↓ 15 % COX activity in AD despite COX subunits were present at normal levels
Bosetti et al., 2002	↓ COX in AD and MCI
Van Zuylen et al., 1992	unchanged
Parker et al., 1990, 1991, 1994	↓ COX in AD

Table 9. Platelet COX activity in AD

[116-119]. It has been suggested that MAO-B activity might be an indicator for severity and clinical progress in AD [57]. In another study, non-psychotic AD patients showed significantly higher platelet MAO-B activity, suggesting that MAO-B activity can differentiate between psychotic and non-psychotic subtypes of AD [56].

References	Effects on MAO-B activity
Zellner et al., 2012	↑ MAO-B activity correlated with ↑ MAO-B protein expression in AD
Mimica et al., 2008	↑ in AD in non-psychotic patients
Soto et al., 1999	↑ 22% MAO-B activity in AD
Götz et al., 1998	↑ MAO-B activity in AD
Bongioanni et al., 1996	↑ MAO-B activity in AD
Ahlkop et al., 1996	unchanged
Konings et al., 1995	unchanged
Parnetti et al., 1992, 1994	↑ MAO-B activity in LOAD compared to controls and EOAD
Regland et al., 1991	↑ MAO-B activity in AD; after B12 therapy MAO-B activity was reduced to a normal level
Schneider et al., 1988	↑ MAO-B activity in female AD without agitation and delusions
Danielczyk et al., 1988	↑ MAO-B activity in AD (non-familial)
Alexopoulos et al., 1987	↑ MAO-B activity in demented patients with and without depression
Adolfsson et al., 1980	↑ MAO-B activity in AD

Table 10. Platelet MAO-B activity in AD

6. Changes of other biological systems

6.1. GSK3β activity

Glycogen synthase kinase 3-beta (GSK3β) is involved in the regulation of glycogen synthesis by phosphorylating and inactivating the glycogen synthase and in the regulation of intracellular signalling pathways [129-131]. Moreover, in neural cell tissues GSK3β is the most important Tau kinase and has been linked to synaptic plasticity and neural injury [132, 133]. Therefore, deregulation of GSK3β has a significant impact on the formation of neurofibrillary tangles. GSK3β is also expressed in platelets and might be involved in platelet activation [134]. A substantial higher expression of GSK3β has been shown in AD and MCI platelets as compared to healthy controls, which also correlated with worse memory performance [135].

6.2. Phospholipase activity

It is known that AD is associated with chronic inflammatory responses and platelets express inflammatory mediators such as chemokines, interleukins, adhesive proteins and contain enzymes such as phospholipase-A2 (PLA2) [20]. Phospholipases are important platelet enzymes involved in the metabolism of membrane phospholipids and inflammatory synthesis. PLA2 and phospholipase C (PLC) are altered in peripheral blood cells and significantly decreased PLC activity in platelets of AD patients has been reported [136], suggesting aberrant phospholipase metabolism. Further, decreased PLA2 activity was found in human platelets [137, 138], which correlates with the degree of cognitive impairment and is modulated by cognitive training in healthy elders [139]. In contrast, an increased platelet PLA2 activity in individuals with AD has also been shown [140]. Thus, again the reports on these enzyme activities are very controversial and do not allow to establish a concluding interpretation.

References	Effects on PLA2 and PLC activity
Krzystanek et al., 2007	↑ PLA2 in AD
Gattaz et al., 1995, 1996, 2004	↓ PLA2 in AD and MCI
Matushima et al., 1995, 1998	↓ PLC in AD

Table 11. Platelet PLA2 and PLC activity in AD

6.3. Overview of other platelet components found to be altered in AD

Several other biological systems have been studied in platelets of AD patients, such as e.g. decreased plasma antioxidant power levels [107], decreased cathepsin D [141], increased platelet glutamine synthetase-like protein level in MCI [112], increased NA,K-ATPase activities in AD [107], unchanged Vitamin E and cholesterol content between AD and controls [32] or increased phenolsulphotransferase activity in demented patients [142] or decreased platelet peripheral-type benzodiazepine binding [143]. However, we do not claim to provide

a complete overview of all markers. Anyhow, none of these markers has been found to be a suitable reproducible marker for AD.

7. Platelets as biomarkers

Biomarkers must objectively reflect physiological processes linked to AD and should be globally reproducible with easy-to-perform tests [144]. Moreover, besides being sensitive enough to differentiate AD from clinically similar diseases, a good biomarker should be able to detect early disease-associated changes. In AD, abnormal metabolism of APP, hyperphosphorylation of Tau, induction of oxidative stress and inflammatory cascades result in pathological changes in liquid fluids. Currently, the laboratory diagnosis of AD is based on the combination of three CSF biomarkers yielding a sensitivity of >95% and specificity of >85% [145, 146]. However, considering the invasiveness of lumbar puncture and the growing incidence rate of AD, the need for biomarkers in more accessible body fluids is necessary. Blood measurements are minimally invasive and less time-consuming compared to CSF. The establishment of new markers in plasma/serum has proven to be difficult because changes mirror a broad spectrum of physiological processes not necessarily related to AD [144]. Moreover, changes in plasma/serum are very small and heterogeneous, thus making the search for reliable and sensitive biomarkers challenging.

Within the search for peripheral biomarkers in AD, blood platelets have been of great interest during the last decades. These anuclear blood cells share several homeostatic functions with neurons such as accumulation and release of neurotransmitters like serotonin, and expression of receptors and enzymes [147]. Moreover, human platelets have shown to be the most important source of more than 90% of the circulating APP [148, 149]. On grounds of these findings, platelets can be considered a valid peripheral model for the analysis of metabolic pathways linked to AD. Despite great efforts, to date no specific platelet biomarker has been successfully established, while some platelet components have shown to be of greater validity than others.

Overall, the so far published studies underline the need to establish concurrent methods in order to yield comparable results and avoid methodological diversity among institutes. Platelets contain several types of surface receptors responding to external stimuli with activation or inhibitory actions [150]. In response to physiological and non-physiological stimuli, platelet granules and their contents are released, thus requiring careful handling during isolation and experimentation in order to avoid activation. Effectively, different platelet isolation and processing procedures may account to a significant extent for the observed inter-rater differences between institutes. Moreover, different phases of the disease, comorbidities or medications need to be taken into consideration when evaluating platelet status in AD patients. Additionally, further analysis and classification of platelets with respect to their different features (e.g. density fraction) might be helpful to generate more significant data. Besides a homogeneous patient group, the selection of healthy, age-matched controls is a known challenge in AD-research and might additionally explain varying data. Therefore, the

so far obtained results need to be replicated in large and homogeneous cohorts according to the same methodological protocol by independent researchers. To date, the use of platelet biomarkers in the diagnostic routine of AD can yet not be recommended and is in urgent need of final evaluation.

8. Conclusions

In conclusion, the search for platelet biomarker so far has highlighted some candidates worth further investigation, while the breakthrough in identifying one satisfyingly specific and sensitive marker has yet to be awaited. Overall, among the studied platelet components and according to the available data, the platelet APP ratio and COX activity seem the most promising candidates for establishing new peripheral biomarkers for AD.

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