ISSN (Online) 2287-3406
Journal of Life Science **2020** Vol. 30. No. 12. 1118~1127 DOI : https://doi.org/10.5352/JLS.2020.30.12.1118

- Review -

Platelets as a Source of Peripheral Aβ Production and Its Potential as a Blood-based Biomarker for Alzheimer's Disease

Jae Seon Kang and Yun-Sik Choi*

Department of Pharmacy, Kyungsung University, Nam-Gu, Busan 48434, Korea

Received November 5, 2020 /Revised December 17, 2020 /Accepted December 18, 2020

Alzheimer's disease causes progressive neuronal loss that leads to cognitive disturbances. It is not currently curable, and there is no way to stop its progression. However, since medical treatment for Alzheimer's disease is most effective in the early stages, early detection can provide the best chance for symptom management. Biomarkers for the diagnosis of Alzheimer's disease include amyloid β (A β) deposition, pathologic tau, and neurodegeneration. Aβ deposition and phosphorylated tau can be detected by cerebrospinal fluid (CSF) analysis or positron emission tomography (PET). However, CSF sampling is quite invasive, and PET analysis needs specialized and expensive equipment. During the last decades, blood-based biomarker analysis has been studied to develop fast and minimally invasive biomarker analysis method. And one of the remarkable findings is the involvement of platelets as a primary source of Aβ in plasma. Aβ can be transported across the blood–brain barrier, creating an equilibrium of Aβ levels between the brain and blood under normal condition. Interestingly, a number of clinical studies have unequivocally demonstrated that plasma Aβ42/Aβ40 ratios are reduced in mild cognitive impairment and Alzheimer's disease. Together, these recent findings may lead to the development of a fast and minimally invasive early diagnostic approach to Alzheimer's disease. In this review, we summarize recent advances in the biomarkers of Alzheimer's disease, especially the involvement of platelets as a source of peripheral Aβ production and its potential as a blood-based biomarker.

Key words : Alzheimer's disease, amyloid β, biomarker, blood, platelet

Introduction

Alzheimer's disease is a neurodegenerative disease and a primary cause of dementia. More than 45 million people worldwide have Alzheimer's disease, and it is estimated that the number of Alzheimer's patients will reach 74.7 million in 2020 and 131 million by 2050[54]. Recent surveys have shown that preventing Alzheimer's disease and preserving cognitive health are among the top concerns of those in the aging public, and many list dementia as their most feared disease—ahead of cancer or stroke [36]. In fact, although the overall death rate from stroke and cardiovascular disease is decreasing, the Alzheimer's-related death rate is increasing in the United States [73]. The major sticking points in overcoming Alzheimer's disease stem from diagnosis and treatment. It is difficult to diagnose Alzheimer's disease in the early stages of the illness, and there is no test to diagnose it definitively before death. There is also a tacit agreement that there is no cure for Alzheimer's disease and that the best strategy is to delay or slow its progression. Further, Alzheimer's disease is a heterogeneous disorder because of idiosyncratic differences in genetic background, environmental triggers, or the presence of other diseases, which makes treatment even more difficult [32].

The symptoms of Alzheimer's disease are quite diverse. Typically, it begins with a mild decline in memory ability and gradually progresses to deteriorating cognitive function and daily activities [41]. By the time Alzheimer's disease is clinically diagnosed, neuronal loss and neuropathologic lesions have occurred in many brain regions [21, 49]. Therefore, to overcome or at least minimize the impact of Alzheimer's disease, we need to discover biomarkers that reflect early symptoms of this disease reliably and reproducibly. Recently, there has been increasing evidence that platelets may be a reliable source of the Alzheimer's biomarker amyloid β (Aβ). Platelets contain high levels of amyloid precursor protein (APP), have enzymatic activities generating Aβ peptides, and have signaling pathways that lead to plate-

***Corresponding author**
Tel : +82-51-663-4890, Fax : +82-51-663-4809
E-mail : tiana@ks.ac.kr

let activation and aggregation which have been described to modulate APP processing [30]. Importantly, there is accumulating evidence showing a correlation between plasma concentrations of Aβ and Alzheimer's dementia. This review summarizes recent advances in the research on Aβ generation by platelets, its relevance, and its potential as a new biomarker of Alzheimer's disease.

Platelets

Platelets, also called thrombocytes, are membrane-bound and -formed elements that are fragments of complete cells. During their development within red marrow, they are derived from a large precursor cell—the megakaryocyte. Platelets consist of cytoplasm surrounded by a cell membrane, and they do not have a nucleus, but they do contain some types of cytoplasmic organelles. These cells have a relatively short life span of 7-10 days in humans, following which they are selectively cleared by the reticuloendothelial system [53]. Although the platelet count (PC) is normally maintained at 150,000~400,000 cells per microliter of blood, chronic inflammation or acute infections can be related to a reactive high PC or a sudden increase/decrease in platelets [44].

Platelet counts in aging and Alzheimer's disease patients

Age-related changes in PCs are still controversial. Stevens and Alexander [68] first examined the PCs of 868 blood donors aged 18-65 and, although age-related change was not found, PCs in women were significantly higher than in men. In the United States, 12,142 inhabitants participated in the Third National Health and Nutrition Examination Survey, in which 60- to 69-year-olds had counts that were $7\times10^3/\mu l$ lower than young adults, and 70- to 90-year-olds had counts that were $18\times10^3/\mu l$ lower [61]. In Italy, PCs in 7,266 inhabitants were examined, and an average platelet decrease of $6\times10^9/l$ for every 10 years of age was observed [4]. Likewise, 18,097 inhabitants participated in the MOLI-SANI project, which showed that a 10-year increase in age corresponds to a sex-adjusted decrease of $10\times10^9/l$ in the PC and that the prevalence of thrombocytopenia increases with age [59].

Further, recent data from 40,987 subjects enrolled in three population-based studies in seven Italian areas—including six geographic isolates—showed that PCs were similar in men and women until the age of 14 but that PCs in old age fell by 35% in men and 25% in women compared with early infancy [5]. Although increasing age is not a direct cause of Alzheimer's disease, it is one of the largest well-known risk factors. Is there, then, any correlation between PCs and Alzheimer's disease? A hospital-based case study in China of 92 Alzheimer's disease patients and 84 age- and sex-matched normal controls revealed no significant differences in PCs [11], and that study was consistent with previous reports. For example, Sevush and colleagues [63] found no difference in overall PCs between 91 patients with probable Alzheimer's disease and 40 age-matched control subjects. Furthermore, an analysis of 20,591 FDA reports on cases of Alzheimer's disease–type dementia found that only 0.4% developed thrombocytopenia, which appears to be associated with long-term use of certain anti-Alzheimer's disease medicines; this data indicates that thrombocytopenia is extremely rare in Alzheimer's disease patient cohorts [34]. Therefore, although PCs decrease with age, altered PCs may not be a direct cause of Alzheimer's disease pathogenesis.

Platelet indices in Alzheimer's disease patients

Platelet indices are considered markers of platelet activation and provide clinical information about various diseases, such as thrombocytopenia and Alzheimer's disease. Clinically important platelet indices include mean platelet volume (MPV), platelet volume distribution width (PDW), plateletcrit (PCT), mean platelet component (MPC), mean platelet mass (MPM), and platelet component distribution width (PCDW). MPV reflects the size of the platelets and is related to platelet production and activation. It has clinical meaning in cardiovascular diseases, respiratory diseases, Crohn's disease, rheumatoid arthritis, diabetes mellitus, and the majority of neoplastic diseases [39]. PDW is a measure of variations in platelet size and may increase when platelets are activated. Both MPV and PDW increase during platelet activation, but PDW is a more specific marker of platelet activation, as MPV increases by simple platelet swelling but PDW does not. Interestingly, clinical investigation data has revealed that Alzheimer's disease patients have lower levels of PDW than normal controls [11, 24, 46, 72].

However, MPV in Alzheimer's disease patients is inconsistent. For example, a clinical report by Chen and colleagues [11] showed increased MPV in Alzheimer's disease patients,

which is consistent with a previous report [38], but other clinical data has shown lower MPVs in patients with vascular dementia and Alzheimer's disease [46, 72]. Since MPV4 is a potential marker of ongoing vascular damage, more studies are needed to verify the involvement of MPV4 in the pathogenesis of Alzheimer's disease.

APP processing in the platelet

Aβ peptides are the main components of the senile plaques that cause Alzheimer's disease and are generated by the proteolytic cleavage of APP. The APP gene produces three major splice variants—APP695, APP751, and APP770—produced in neurons, endothelial cells, and platelets, respectively [67]. APP751 and APP770 are also expressed in endothelial cells, and these expression levels are higher in the endothelial cells of cerebral blood vessels than in peripheral arteries [25]. As shown in Fig. 1, APP is cleaved by an α-secretase, producing soluble APPα (sAPPα) and a membrane-tethered α-C terminal fragment (CTF83). APP can also be cleaved by a β-secretase, producing soluble APPβ (sAPPβ) and a β-C terminal fragment (CTF99). CTF99 is then further cleaved by ɣ-secretase, which liberates the Aβ peptides $Aβ_{40}$ and $Aβ_{42}$ [13]. Consequently, when APP is cleaved by α-secretase first, Aβ peptides are not produced.

Platelets are small anucleate blood cells and contain diverse granules, such as α-granules, dense granules, and lysosomes. Platelet α-granules have APP, and APP-processing enzymes, such as α-, β-, and ɣ-secretases, are also found in platelets [3, 16, 69]. In addition, platelets can produce all APP fragments found in neurons: the soluble secretory APPs (sAPPα and sAPPβ); the amyloidogenic fragments CTF99 and CTF83; and the Aβ peptide [28]. Although α-secretase activity is the dominant pathway in platelets under normal

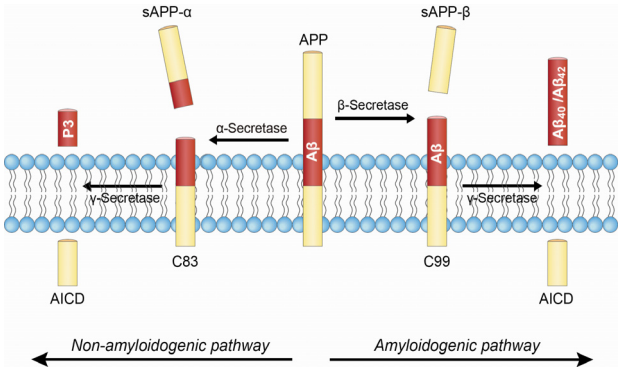

Fig. 1. Amyloid precursor protein (APP) processing pathways.

conditions, both sAPP and Aβ can be released from platelets in response to thrombin and collagen, which induce platelet degranulation [45]. Therefore, platelets have all the machinery to produce APP fragments, and it is believed that APP fragments from platelets play a role in the normal function and pathogenesis of blood vessels.

Platelets as a source of amyloidogenic amyloid β in the blood

As mentioned above, platelet α-granules contain APP and release their contents when the platelet is activated. One compelling piece of evidence was found by Van Nostrand and colleagues [70], in which the activation of platelets with either collagen or thrombin resulted in the secretion of approximately 46% or 53% of total APP, respectively. Of note is that platelets are the primary source (~90%) of Aβ peptide in the blood [12]. In addition, activated platelets in those with advanced Alzheimer's disease contain significantly higher amounts of surface membrane‐bound APP than platelets from non-demented age-matched individuals [18]. Further, in the platelets of Alzheimer's disease patients, increased activation of β-secretase (BACE1) and decreased activation of α-secretase (ADAM10) was observed [16, 50, 69].

Since α- and β-secretase pathways seem to be mutually exclusive, reduction of α-secretase activity can enhance β-secretase amyloidogenic cleavage of APP. Along the same lines, the content of APP fragments metabolized by α-secretase (αAPP) in platelets from Alzheimer's disease patients was found to be significantly lower than from control subjects, and this phenomenon is consistent findings from the cerebrospinal fluid of Alzheimer's disease patients [16]. Interestingly, Aβ peptides can also be generated by cleaving the platelet-released APP in brain vessels' endothelial cells [17]. Combined, these reports suggest that platelets are the main source of amyloidogenic Aβ in the blood of Alzheimer's disease patients.

Transport of peripheral amyloid β into the brain

Abnormal Aβ accumulation in the brain is responsible for the neurodegeneration and cognitive decline observed in Alzheimer's disease patients. Its accumulation is caused by either overproduction of Aβ or a dysfunction of Aβ clearance, and Aβ clearance may be affected by the concentration equilibrium between the brain and periphery (influx or ef-

flux of Aβ). Interestingly, dysfunction of Aβ clearance is hypothetically estimated in 99% of all Alzheimer's disease patients. Aβ efflux mechanisms include the blood–brain barrier (BBB), lymphatic-related, and arachnoid granule pathways [14]. In contrast, recent noteworthy research has shown that Aβ interaction with receptors for advanced glycation end products (RAGE) in the blood vessels result in the transport of Aβ across the BBB and that RAGE-ligand interaction suppresses accumulation of Aβ in the brain parenchyma [20].

Interestingly, RAGE mediates the continuous influx of peripheral Aβ into the brain but cannot clear brain-derived Aβ [65]. Supporting this theory, constant transfusion of blood from APPswe/PS1dE9 mice to their wild-type littermates demonstrated that the human Aβ originating from transgenic Alzheimer's disease model mice enters the circulation and accumulates in the brains of wild-type mice, forming cerebral amyloid angiopathy and Aβ plaques after a 12-month period of parabiosis [7]. Likewise, FPS-ZM1, a RAGE-specific blocking agent, inhibits both Aβ influx across the BBB and RAGE expression, reducing hippocampal Aβ levels and reversing memory impairment in db/db mice [71].

These innate and exquisite influx and efflux machineries maintain a balance of Aβ levels between the central nervous system and periphery under normal conditions. Interestingly, although the balance between the influx and efflux of Aβ through the BBB is maintained while young, Aβ efflux is significantly increased in older genetic animal models of Alzheimer's disease [23]. Another line of evidence has shown that there is a dynamic equilibrium of Aβ levels between the central nervous system and plasma until the age when Aβ deposition declines, at which point plaque formation creates a new kinetics of Aβ flow because soluble Aβ from the central nervous system not only enters the plasma, but also deposits onto the amyloid plaques in the central nervous system [22]. Combined, there is a well-maintained balance of Aβ levels between the central nervous system and blood, and the disruption of the balance or a kinetic shift based on the concentration of plasma Aβ may precede or run parallel with the pathogenesis of Alzheimer's disease.

Amyloid β as a peripheral biomarker of Alzheimer's disease

Biomarkers are indices that represent what is happening inside our bodies and can be found by laboratory and clinical tests. Biomarkers can help doctors and scientists diagnose diseases and health conditions, identify health risks, monitor responses to treatment, and see how a person's disease or health condition changes over time. The National Institute on Aging and Alzheimer's Association research framework defined Alzheimer's disease by its underlying pathologic processes, which can be documented by post-mortem examination or *in vivo* by biomarkers [35]. Biomarkers in Alzheimer's disease are classified with the AT(N) system—Aβ deposition, pathologic tau, and neurodegeneration. Aβ deposition includes $A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$ ratios in the CSF and amyloid positron emission tomography (PET); aggregated tau includes phosphorylated tau in the CSF and Tau PET; and neurodegeneration includes anatomic MRI, fluorodeoxyglucose (FDG) PET, and total tau in the CSF [35].

Biomarker analysis from CSF and PET data correlates highly with brain biopsy findings, and changes enable early diagnosis of Alzheimer's disease [43, 62, 74]. However, Alzheimer's is a heterogeneous disorder; thus, in many cases, a single biomarker is not accurate enough to diagnose disease status correctly. In addition, although serious complications are rare, CSF-based biomarker analysis needs lumbar puncture, and this approach is quite invasive. In the case of PET analysis, patients have to visit a center equipped with PET, and running costs are high. Therefore, the medical need is growing for non-invasive and cost-saving biomarker analysis methods for Alzheimer's disease diagnosis that are accurate, sensitive, and reproducible.

Could Aβ in the blood be a biomarker of Alzheimer's disease? There is accumulating clinical evidence supporting the theory wherein peripheral Aβ levels are such a biomarker. First, as described above, there is a dynamic equilibrium of Aβ levels between the central nervous system and plasma, and there is thus a strong correlation between Aβ levels in the blood and neuropathological changes in the central nervous system [52]. Interestingly, the equilibrium of Aβ levels between the central nervous system and plasma is maintained until the age when Aβ deposition declines, at which point plaque formation creates a new kinetics of Aβ flow because soluble Aβ from the central nervous system not only enters the plasma, but also deposits onto amyloid plaques in the central nervous system [22].

Along these lines, a number of studies have suggested that the $A\beta_{42}/A\beta_{40}$ ratio is significantly reduced in patients with Alzheimer's disease and mild cognitive impairment [1, 6, 15, 29, 31, 37, 40, 42, 55-57, 60, 64]. One outstanding piece

of evidence suggesting an association between a decreased $A\beta_{42}$/ $A\beta_{40}$ ratio, mild cognitive impairment, and Alzheimer's disease came from a prospective study by Graff-Radford et al. [31]. They measured plasma $A\beta_{40}$ and $A\beta_{42}$ levels from 563 cognitively normal volunteers and followed up for between 2 and 12 years. In that study, 53 subjects developed mild cognitive impairment or Alzheimer's disease, and subjects with low plasma $A\beta_{42}/A\beta_{40}$ ratios were at significantly greater risk. Furthermore, Okereke et al. [55] measured the plasma $A\beta_{40}$ and $A\beta_{42}$ levels of 481 participants in late midlife (mean age 63.6 years), and cognitive testing was conducted 10 years later. In their study, lower plasma $A\beta_{42}/A\beta_{40}$ ratios were associated with worse late-life decline in cognitive functions. Combined, these reports suggest that plasma $A\beta_{42}/A\beta_{40}$ ratios may be a clinically applicable premorbid biomarker for screening elderly subjects who are at potentially higher risk for developing mild cognitive impairment or Alzheimer's disease.

Advantages and disadvantages of plasma $A\beta_{42}/A\beta_{40}$ as a biomarker of Alzheimer's disease

As described above, CSF-based biomarker analysis requires a rather invasive procedure—lumbar puncture—and for a PET analysis, patients must visit a center equipped with PET, whose running costs are high. In contrast, blood biomarkers can be analyzed by drawing small amounts of blood, and this procedure is non or minimally invasive. Blood testing is a well-established clinical procedure worldwide, so no further training is needed, and drawing blood is relatively cheap. However, the most important advantage is that periodic blood draws and biomarker analysis would enable elderly people to monitor the potential risk of mild cognitive impairment or Alzheimer's disease in advance. This is important because Alzheimer's disease is not currently curable, and its care requires early diagnosis and multidisciplinary management.

However, there are also some disadvantages to using plasma $A\beta_{42}/A\beta_{40}$ ratios as a biomarker. First, the concentration of Aβ in the blood is much lower than in the CSF (10-fold lower in plasma than in CSF). In addition, blood contains cells and different molecules, such as protein, nucleic acids, lipids, and metabolites, and this complexity may provide variability between analyses. Further, physiological status and health conditions, such as inflammatory and metabolic disorders, can derange the composition of blood components, which may make blood testing unreliable [33]. Nonetheless, blood-based biomarkers would be an ideal option as the first-step of a multi-stage diagnostic process and provide the means to determine which individuals or patients should receive referral for assessment by specialists, including diagnostic CSF analysis, magnetic resonance imaging (MRI), or amyloid PET diagnostics [33].

Other sources of amyloid β and its roles in the peripheries

In the brain, although glial cells have the means to produce Aβ peptides, it is mostly synthesized by neurons [58]. Likewise, platelets contribute to 90~95% of circulating amyloid peptides in our body [26, 66]. In the periphery there are other sources of Aβ peptides which play an important role in pathophysiology. For example, hyperglycemia increases expression of full-length APP accompanied by increased secretion of $A\beta_{42}$ leading to decreased endothelial tight junction [10]. In addition, ischemia, cellular stress or inflammation increases cell surface localization of APP in endothelial cells and this event may contribute to the impaired homeostasis of Aβ clearance from the brain [8, 26, 51]. Interestingly, deposit of $A\beta_{42}$ in capillaries highly correlated with both $A\beta_{42}$ deposits in plaques and morphological Alzheimer's disease criteria [2]. Further, Aβ exerts antifibrotic function by both autocrine and paracrine manners on hepatic stellate cells and liver sinusoidal endothelial cells by suppressing TGF-β release and elevating NO production [9]. Combined, although platelets are major source of amyloid peptides in the periphery, amyloid peptides from other types of cells also participate in the pathogenesis of diseases including Alzheimer's disease.

Suggestive outline from generation of Aβ in platelets to Alzheimer's disease pathogenesis

There are two types of Aβ peptides transports across the blood brain barrier, influx into the brain and clearance out of the brain. First, Aβ peptides secreted into the blood can be transported into the brain by RAGE [20]. On the other hand, LDL receptor-related proteins (LRP) mediates clearance of Aβ peptides [19]. Under normal condition, influx and efflux machineries maintain a balance of Aβ levels be-

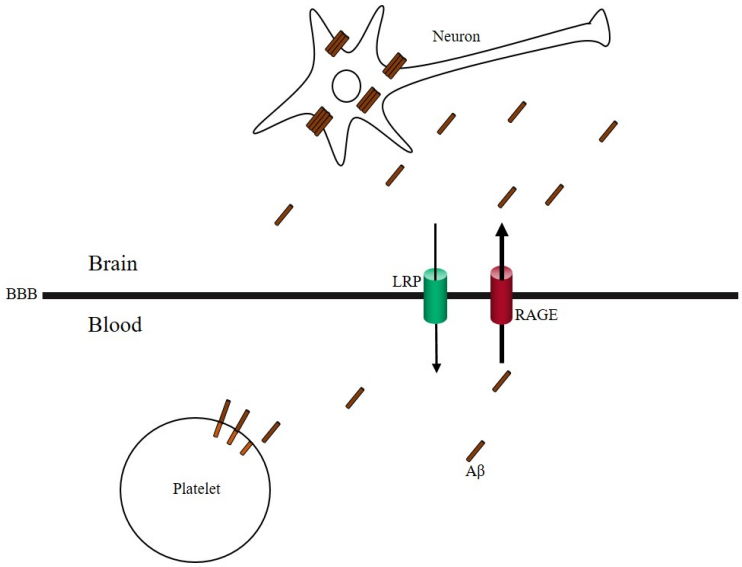

Fig. 2. Plasma Aβ level is increased in certain conditions such as mild cognitive impairment, Alzheimer's disease or increased membrane cholesterol content in platelets. These events may disrupt the balance of Aβ concentration between brain and plasma, and lead increased influx of Aβ peptides into the brain resulting in Aβ plaque formation. RAGE: receptors for advanced glycation end products, LRP: LDL receptor-related proteins, A β: amyloid β, BBB: blood-brain barrier.

tween the brain and periphery. However, as shown in Fig. 2, platelet β-secretase activity is elevated in mild cognitive impairment or Alzheimer's disease [27, 48] and membrane cholesterol content in platelets positively correlates with β -secretase activity [47]. Thus, when Aβ production in the platelets increases, the balance of Aβ levels may be disrupted or a kinetic shift could favor Aβ plaque formation inside the brain leading cognitive impairment as well as Alzheimer's disease.

Conclusion and future focuses

In the last decade, great scientific advances have been made in the field of Alzheimer's disease. Elaborate and prospective studies have revealed its pathogenesis, and key molecules have been developed as biomarkers. However, unfortunately, the currently established biomarkers are key molecules from CSF and analysis by PET. Sampling of CSF is an invasive procedure, and PET analysis needs expensive equipment. Therefore, a fast, non-invasive, and cost-saving biomarker analysis method is needed, and a validated blood-based biomarker analysis could be the answer.

As shown above, low $A\beta_{42}/A\beta_{40}$ ratios in the blood are correlated with the pathogenesis of Alzheimer's disease and cognitive decline. In addition, prospective studies in elderly people have shown that a low $A\beta_{42}/A\beta_{40}$ ratio represents a high risk of Alzheimer's disease progression. Combined, plasma $A\beta_{42}/A\beta_{40}$ ratios may be a credible biomarker of Alzheimer's disease and mild cognitive impairment. In a clinical situation, periodic analysis of plasma $A\beta_{42}/A\beta_{40}$ ratios may be useful in monitoring the potential for cognitive decline in elderly people and could be also used as a precedent analysis for Alzheimer's disease. Diagnosis with a blood test in primary care could thus provide access to confirmatory diagnosis with PET or CSF sampling. In the future the better characterization of the biological and pathophysiological role of Aβ generated by platelets will open a new chapter on early diagnosis of Alzheimer's disease and development of new treatment option. Clinically, plasma Aβ level will prompt clinicians to diagnose Alzheimer's disease patients before its symptoms are transparently overt. In parallel, it is expected that additional research is performed to clearly elucidate the therapeutic potential of approaches targeting plasma Aβ. For example, molecules capturing Aβ in the plasma or inhibiting the function of RAGE may provide benefits in preventing or delaying the progression of Alzheimer's disease. For this purposes, since the main source of Aβ in the blood is the platelets, more studies will be needed to elucidate the link between the production of amyloidogenic Aβ in the platelets and its potential as a therapeutic target against Alzheimer's disease.

Acknowledgements

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI18C2383).

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Abdullah, L., Luis, C., Paris, D., Mouzon, B., Ait-Ghezala, G., Keegan, A. P., Wang, D., Crawford, F. and Mullan, M. 2009. Serum Abeta levels as predictors of conversion to mild cognitive impairment/Alzheimer disease in an ADAPT subcohort. *Mol. Med.* **15**, 432-437.
2. Attems, J., Lintner, F. and Jellinger, K. A. 2004. Amyloid beta peptide 1-42 highly correlates with capillary cerebral amyloid angiopathy and Alzheimer disease pathology. *Acta Neuropathol.* **107**, 283-291.
3. Bermejo-Bescós, P., Martín-Aragón, S., Jiménez-Aliaga, K., Benedí, J., Felici, E., Gil, P., Ribera, J. M. and Villar, A. M. 2013. Processing of the platelet amyloid precursor protein in the mild cognitive impairment (MCI). *Neurochem. Res.* **38**, 1415-1423.
4. Biino, G., Gasparini, P., D'Adamo, P., Ciullo, M., Nutile, T., Toniolo, D., Sala, C., Minelli, C., Gögele, M. and Balduini, C, L. 2012. Influence of age, sex and ethnicity on platelet count in five Italian geographic isolates: mild thrombocytopenia may be physiological. *Br. J. Haematol.* **157**, 384-387.
5. Biino, G., Santimone, I., Minelli, C., Sorice, R., Frongia, B., Traglia, M., Ulivi, S., Di Castelnuovo, A., Gögele, M., Nutile, T., Francavilla, M., Sala, C., Pirastu, N., Cerletti, C., Iacoviello, L., Gasparini, P., Toniolo, D., Ciullo, M., Pramstaller, P., Pirastu, M., de Gaetano, G. and Balduini, C. L. 2013. Age- and sex-related variations in platelet count in Italy: a proposal of reference ranges based on 40987 subjects' data. *PLoS One* **8**, e54289.
6. Blennow, K., De Meyer, G., Hansson, O., Minthon, L., Wallin, A., Zetterberg, H., Lewczuk, P., Vanderstichele, H., Vanmechelen, E., Kornhuber, J., Wiltfang, J.; KND-Study Group, Heuser, I., Maier, W., Luckhaus, C., Rüther, E., Hüll, M., Jahn, H., Gertz, H. J., Frölich, L., Hampel, H. and Pernetzki, R. 2009. Evolution of Abeta42 and Abeta40 levels and Abeta42/Abeta40 ratio in plasma during progression of Alzheimer's disease: a multicenter assessment. *J. Nutr. Health Aging* **13**, 205-208.
7. Bu, X. L., Xiang, Y., Jin, W. S., Wang, J., Shen, L. L., Huang, Z. L., Zhang, K., Liu, Y. H., Zeng, F., Liu, J. H., Sun, H. L., Zhuang, Z. Q., Chen, S. H., Yao, X. Q., Giunta, B., Shan, Y. C., Tan, J., Chen, X. W., Dong, Z. F., Zhou, H. D., Zhou, X. F., Song, W. and Wang, Y. J. 2018. Blood-derived amyloid-β protein induces Alzheimer's disease pathologies. *Mol. Psychiatry* **23**, 1948-1956.
8. Bulbarelli, A., Lonati, E., Brambilla, A., Orlando, A., Cazzaniga, E., Piazza, F., Ferrarese, C., Masserini, M. and Sancini, G. 2012. Aβ42 production in brain capillary endothelial cells after oxygen and glucose deprivation. *Mol. Cell Neurosci.* **49**, 415-422.
9. Buniatian, G. H., Hartmann, H. J., Traub, P., Wiesinger, H., Albinus, M., Nagel, W., Shoeman, R., Mecke, D. and Weser, U. 2002. Glial fibrillary acidic protein-positive cells of the kidney are capable of raising a protective biochemical barrier similar to astrocytes: expression of metallothionein in podocytes. *Anat. Rec.* **267**, 296-306.
10. Chao, A. C., Lee, T. C., Juo, S. H. and Yang, D. I. 2016. Hyperglycemia increases the production of amyloid beta-peptide leading to decreased endothelial tight junction. *CNS Neurosci. Ther.* **22**, 291-297
11. Chen, S. H., Bu, X. L., Jin, W. S., Shen, L. L., Wang, J., Zhuang, Z. Q., Zhang, T., Zeng, F., Yao, X. Q., Zhou, H. D. and Wang, Y. J. 2017. Altered peripheral profile of blood cells in Alzheimer disease: A hospital-based case-control study. *Medicine (Baltimore)* **96**, e6843.
12. Chen, M., Inestrosa, N. C., Ross, G. S. and Fernandez, H. L. 1995. Platelets are the primary source of amyloid beta-peptide in human blood. *Biochem. Biophys. Res. Commun.* **213**, 96-103.
13. Chen, G. F., Xu, T. H., Yan, Y., Zhou, Y. R., Jiang, Y., Melcher, K. and Xu, H. E. 2017. Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol. Sin.* **38**, 1205-1235.
14. Cheng, Y., Tian, D. Y. and Wang, Y. J. 2020. Peripheral clearance of brain-derived Aβ in Alzheimer's disease: pathophysiology and therapeutic perspectives. *Transl. Neurodegener.* **9**, 16.
15. Chouraki, V., Beiser, A., Younkin, L., Preis, S. R., Weinstein, G., Hansson, O., Skoog, I., Lambert, J. C., Au, R., Launer, L., Wolf, P. A., Younkin, S. and Seshadri, S. 2015. Plasma amyloid-β and risk of Alzheimer's disease in the Framingham Heart Study. *Alzheimers Dement.* **11**, 249-257.
16. Colciaghi, F., Borroni, B., Pastorino, L., Marcello, E., Zimmermann, M., Cattabeni, F., Padovani, A. and Di Luca, M. 2002. *α*-Secretase ADAM10 as well as *α*APPs is reduced in platelets and CSF of Alzheimer disease patients. *Mol. Med.* **8**, 67-74.
17. Davies, T. A., Billingslea, A. M., Long, H. J., Tibbles, H., Wells, J. M., Eisenhauer, P. B., Smith, S. J., Cribbs, D. H., Fine, R. E. and Simons, E. R. 1998. Brain endothelial cell enzymes cleave platelet-retained amyloid precursor protein. *J. Lab. Clin. Med.* **132**, 341-350.
18. Davies, T. A., Long, H. J., Sgro, K., Rathbun, W. H., Mc Menamin, M. E., Seetoo, K., Tibbles, H., Billingslea, A. M., Fine, R. E., Fishman, J. B., Levesque, C. A., Smith, S. J., Wells, J. M. and Simons, E. R. 1997. Activated Alzheimer disease platelets retain more beta amyloid precursor protein. *Neurobiol. Aging* **18**, 147-153.
19. Deane, R., Bell, R. D., Sagare, A. and Zlokovic, B. V. 2009. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol. Disord. Drug Targets* **8**, 16-30.
20. Deane, R., Du Yan, S., Submamaryan, R. K., LaRue, B., Jovanovic, S., Hogg, E., Welch, D., Manness, L., Lin, C., Yu, J., Zhu, H., Ghiso, J., Frangione, B., Stern, A., Schmidt, A. M., Armstrong, D. L., Arnold, B., Liliensiek, B., Nawroth,

P., Hofman, F., Kindy, M., Stern, D. and Zlokovic, B. 2003. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat. Med.* **9**, 907-913.
21. DeKosky, S. T. and Marek, K. 2003. Looking backward to move forward: early detection of neurodegenerative disorders. *Science* **302**, 830-834.
22. DeMattos, R. B., Bales, K. R., Parsadanian, M., O'Dell, M. A., Foss, E. M., Paul, S. M. and Holtzman, D. M. 2002. Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. *J. Neurochem.* **81**, 229-236.
23. Do, T. M., Dodacki, A., Alata, W., Calon, F., Nicolic, S., Scherrmann, J. M., Farinotti, R. and Bourasset, F. 2016. Age-dependent regulation of the blood-brain barrier influx/efflux equilibrium of amyloid-β peptide in a mouse model of Alzheimer's disease (3xTg-AD). *J. Alzheimers Dis.* **49**, 287-300.
24. Dong, X., Nao, J., Shi, J. and Zheng, D. 2019. Predictive value of routine peripheral blood biomarkers in Alzheimer's disease. *Front. Aging Neurosci.* **11**, 332.
25. d'Uscio, L. V., He, T. and Katusic, Z. S. 2017. Expression and processing of amyloid precursor protein in vascular endothelium. *Physiology (Bethesda)* **32**, 20-32.
26. d'Uscio, L. V. and Katusic, Z. S. 2019. Vascular phenotype of amyloid precursor protein-deficient mice. *Am. J. Physiol. Heart Circ. Physiol.* **316**, H1297-H1308.
27. Evin, G. and Li, Q. X. 2012. Platelets and Alzheimer's disease: Potential of APP as a biomarker. *World J. Psychiatry* **2**, 102-113.
28. Evin, G., Zhu, A., Holsinger, R. M., Masters, C. L. and Li, Q. X. 2003. Proteolytic processing of the Alzheimer's disease amyloid precursor protein in brain and platelets. *J. Neurosci. Res.* **74**, 386-392.
29. Fandos, N., Pérez-Grijalba, V., Pesini, P., Olmos, S., Bossa, M., Villemagne, V. L., Doecke, J., Fowler, C., Masters, C. L. and Sarasa, M. AIBL Research Group. 2017. Plasma amyloid β 42/40 ratios as biomarkers for amyloid β cerebral deposition in cognitively normal individuals. *Alzheimers Dement (Amst).* **8**, 179-187.
30. Gowert, N. S., Donner, L., Chatterjee, M., Eisele, Y. S., Towhid, S. T., Münzer, P., Walker, B., Ogorek, I., Borst, O., Grandoch, M., Schaller, M., Fischer, J. W., Gawaz, M., Weggen, S., Lang, F., Jucker, M. and Elvers, M. 2014. Blood platelets in the progression of Alzheimer's disease. *PLoS One* **9**, e90523.
31. Graff-Radford, N. R., Crook, J. E., Lucas, J., Boeve, B. F., Knopman, D. S., Ivnik, R. J., Smith, G. E., Younkin, L. H., Petersen, R. C. and Younkin, S. G. 2007. Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. *Arch. Neurol.* **64**, 354-362.
32. Guest, F. L., Rahmoune, H. and Guest, P. C. 2020. Early diagnosis and targeted treatment strategy for improved therapeutic outcomes in Alzheimer's disease. *Adv. Exp. Med. Biol.* **1260**, 175-191.
33. Hampel, H., O'Bryant, S. E., Molinuevo, J. L., Zetterberg, H., Masters, C. L., Lista, S., Kiddle, S. J., Batrla, R. and Blennow, K. 2018. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat. Rev. Neurol.* **14**, 639-652.
34. Inyushin, M. Y., Sanabria, P., Rojas, L., Kucheryavykh, Y. and Kucheryavykh, L. 2017. Aβ peptide originated from platelets promises new strategy in anti-Alzheimer's drug development. *Biomed. Res. Int.* **2017**, 3948360.
35. Jack, C. R. Jr, Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M. and Sperling, R. 2018. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* **14**, 535-562.
36. Kelley, M., Ulin, B. and McGuire, L. C. 2018. Reducing the risk of Alzheimer's disease and maintaining brain health in an aging society. *Public Health Rep.* **133**, 225-229.
37. Kim, H. J., Park, K. W., Kim, T. E., Im, J. Y., Shin, H. S., Kim, S., Lee, D. H., Ye, B. S., Kim, J. H., Kim, E. J., Park, K. H., Han, H. J., Jeong, J. H., Choi, S. H. and Park, S. A. 2015. Elevation of the plasma Aβ40/Aβ42 ratio as a diagnostic marker of sporadic early-onset Alzheimer's disease. *J. Alzheimers Dis.* **48**, 1043-1050.
38. Koç, E. R., Uzar, E., Çirak, Y., Parlak Demir, Y. and Ilhan, A. 2014. The increase of mean platelet volume in patients with Alzheimer disease. *Turk. J. Med. Sci.* **44**, 1060-1066.
39. Korniluk, A., Koper-Lenkiewicz, O. M., Kamińska, J., Kemona, H. and Dymicka-Piekarska, V. 2019. Mean Platelet Volume (MPV): New perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediators Inflamm.* **2019**, 9213074.
40. Koyama, A., Okereke, O. I., Yang, T., Blacker, D., Selkoe, D. J. and Grodstein, F. 2012. Plasma amyloid-β as a predictor of dementia and cognitive decline: a systematic review and meta-analysis. *Arch. Neurol.* **69**, 824-831.
41. Kukull, W. A. and Bowen, J. D. 2002. Dementia epidemiology. *Med. Clin. North Am.* **86**, 573-590.
42. Lambert, J. C., Schraen-Maschke, S., Richard, F., Fievet, N., Rouaud, O., Berr, C., Dartigues, J. F., Tzourio, C., Alpérovitch, A., Buée, L. and Amouyel, P. 2009. Association of plasma amyloid beta with risk of dementia: the prospective Three-City Study. *Neurology* **73**, 847-853.
43. Lewczuk, P. and Kornhuber, J. 2016. Do we still need positron emission tomography for early Alzheimer's disease diagnosis? *Brain* **139**, e60.
44. Li, R., Hoffmeister, K. M. and Falet, H. 2016. Glycans and the platelet life cycle. *Platelets* **27**, 505-511.
45. Li, Q. X., Whyte, S., Tanner, J. E., Evin, G., Beyreuther, K. and Masters, C. L. 1998. Secretion of Alzheimer's disease Abeta amyloid peptide by activated human platelets. *Lab. Invest.* **78**, 461-469.
46. Liang, Q. C., Jin, D., Li, Y. and Wang, R. T. 2014. Mean platelet volume and platelet distribution width in vascular dementia and Alzheimer's disease. *Platelets* **25**, 433-438.

47. Liu, W. W., Todd, S., Coulson, D. T., Irvine, G. B., Passmore, A. P., McGuinness, B., McConville, M., Craig, D. and Johnston, J. A. 2009. A novel reciprocal and biphasic relationship between membrane cholesterol and beta-secretase activity in SH-SY5Y cells and in human platelets. *J. Neurochem.* **108**, 341-249.
48. Liu, W. W., Todd, S., Craig, D., Passmore, A. P., Coulson, D. T., Murphy, S., Irvine, G. B. and Johnston, J. A. 2007. Elevated platelet beta-secretase activity in mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* **24**, 464-468.
49. Mantzavinos, V. and Alexiou, A. 2017. Biomarkers for Alzheimer's disease diagnosis. *Curr. Alzheimer Res.* **14**, 1149-1154.
50. McGuinness, B., Fuchs, M., Barrett, S. L., Passmore, A. P. and Johnston, J. A. 2016. Platelet membrane β-secretase activity in mild cognitive impairment and conversion to dementia: a longitudinal study. *J. Alzheimers Dis.* **49**, 1095-1103.
51. Muche, A., Bürger, S., Arendt, T. and Schliebs, R. 2015. Hypoxic stress, brain vascular system, and β-amyloid: a primary cell culture study. *Nutr. Neurosci.* **18**, 1-11.
52. Nakamura, A., Kaneko, N., Villemagne, V. L., Kato, T., Doecke, J., Doré, V., Fowler, C., Li, Q. X., Martins, R., Rowe, C., Tomita, T., Matsuzaki, K., Ishii, K., Ishii, K., Arahata, Y., Iwamoto, S., Ito, K., Tanaka, K., Masters, C. L. and Yanagisawa, K. 2018. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* **554**, 249-254.
53. Nayak, M. K., Kulkarni, P. P. and Dash, D. 2013. Regulatory role of proteasome in determination of platelet life span. *J. Biol. Chem.* **288**, 6826-6834.
54. Nguyen, K. V. 2018. Special Issue: Alzheimer's disease. *AIMS Neurosci.* **5**, 74-80.
55. Okereke, O. I., Xia, W., Selkoe, D. J. and Grodstein, F. 2009. Ten-year change in plasma amyloid beta levels and late-life cognitive decline. *Arch. Neurol.* **66**, 1247-1253.
56. Ovod, V., Ramsey, K. N., Mawuenyega, K. G., Bollinger, J. G., Hicks, T., Schneider, T., Sullivan, M., Paumier, K., Holtzman, D. M., Morris, J. C., Benzinger, T., Fagan, A. M., Patterson, B. W. and Bateman, R. J. 2017. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement.* **13**, 841-849.
57. Pérez-Grijalba, V., Romero, J., Pesini, P., Sarasa, L., Monleón, I., San-José, I., Arbizu, J., Martínez-Lage, P., Munuera, J., Ruiz, A., Tárraga, L., Boada, M. and Sarasa, M. 2019. Plasma Aβ42/40 ratio detects early stages of Alzheimer's disease and correlates with CSF and neuroimaging biomarkers in the AB255 study. *J. Prev. Alzheimers Dis.* **6**, 34-41.
58. Roher, A. E., Esh, C. L., Kokjohn, T. A., Castaño, E. M., Van Vickle, G. D., Kalback, W. M., Patton, R. L., Luehrs, D. C., Daugs, I. D., Kuo, Y. M., Emmerling, M. R., Soares, H., Quinn, J. F., Kaye, J., Connor, D. J., Silverberg, N. B., Adler, C. H., Seward, J. D., Beach, T. G. and Sabbagh, M. N. 2009. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement.* **5**, 18-29.
59. Santimone, I., Di Castelnuovo, A., De Curtis, A., Spinelli, M., Cugino, D., Gianfagna, F., Zito, F., Donati, M. B., Cerletti, C., de Gaetano, G., Iacoviello, L. and MOLI-SANI Project Investigators. 2011. White blood cell count, sex and age are major determinants of heterogeneity of platelet indices in an adult general population: results from the MOLI-SANI project. *Haematologica* **96**, 1180-1188.
60. Schupf, N., Tang, M. X., Fukuyama, H., Manly, J., Andrews, H., Mehta, P., Ravetch, J. and Mayeux, R. 2008. Peripheral Abeta subspecies as risk biomarkers of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA.* **105**, 14052-14057.
61. Segal, J. B. and Moliterno, A. R. 2006. Platelet counts differ by sex, ethnicity, and age in the United States. *Ann. Epidemiol.* **16**, 123-130.
62. Seppälä, T. T., Nerg, O., Koivisto, A. M., Rummukainen, J., Puli, L., Zetterberg, H., Pyykkö, O. T., Helisalmi, S., Alafuzoff, I., Hiltunen, M., Jääskeläinen, J. E., Rinne, J., Soininen, H., Leinonen, V. and Herukka, S. K. 2012. CSF biomarkers for Alzheimer disease correlate with cortical brain biopsy findings. *Neurology* **78**, 1568-1575.
63. Sevush, S., Jy, W., Horstman, L. L., Mao, W. W., Kolodny, L. and Ahn, Y. S. 1998. Platelet activation in Alzheimer disease. *Arch. Neurol.* **55**, 530-536.
64. Shahpasand-Kroner, H., Klafki, H. W., Bauer, C., Schuchhardt, J., Hüttenrauch, M., Stazi, M., Bouter, C., Wirths, O., Vogelgsang, J. and Wiltfang, J. 2018. A two-step immunoassay for the simultaneous assessment of Aβ38, Aβ40 and Aβ42 in human blood plasma supports the Aβ42/Aβ40 ratio as a promising biomarker candidate of Alzheimer's disease. *Alzheimers Res. Ther.* **10**, 121.
65. Shibata, M., Yamada, S., Kumar, S. R., Calero, M., Bading, J., Frangione, B., Holtzman, D. M., Miller, C. A., Strickland, D. K., Ghiso, J. and Zlokovic, B. V. 2000. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J. Clin. Invest.* **106**, 1489-1499.
66. Sonkar, V. K., Kulkarni, P. P. and Dash, D. 2014. Amyloid β peptide stimulates platelet activation through RhoA-dependent modulation of actomyosin organization. *FASEB J.* **28**, 1819-1829.
67. Stakos, D. A., Stamatelopoulos, K., Bampatsias, D., Sachse, M., Zormpas, E., Vlachogiannis, N. I., Tual-Chalot, S. and Stellos, K. 2020. The Alzheimer's Disease Amyloid-beta hypothesis in cardiovascular aging and disease: JACC Focus Seminar. *J. Am. Coll. Cardiol.* **75**, 952-967.
68. Stevens, R. F. and Alexander, M. K. 1977. A sex difference in the platelet count. *Br. J. Haematol.* **37**, 295-300.
69. Tang, K., Hynan, L. S., Baskin, F. and Rosenberg, R. N. 2006. Platelet amyloid precursor protein processing: a bio-marker for Alzheimer's disease. *J. Neurol. Sci.* **240**, 53-58.
70. Van Nostrand, W. E., Schmaier, A. H., Farrow, J. S. and Cunningham, D. D. 1990. Protease nexin-II (amyloid beta-protein precursor): a platelet alpha-granule protein. *Science* **248**, 745-748.
71. Wang, H., Chen, F., Du, Y. F., Long, Y., Reed, M. N., Hu, M., Suppiramaniam, V., Hong, H. and Tang, S. S. 2018. Targeted inhibition of RAGE reduces amyloid-β influx

across the blood-brain barrier and improves cognitive deficits in db/db mice. *Neuropharmacology* **131**, 143-153.
72. Wang, R. T., Jin, D., Li, Y. and Liang, Q. C. 2013. Decreased mean platelet volume and platelet distribution width are associated with mild cognitive impairment and Alzheimer's disease. *J. Psychiatr. Res.* **47**, 644-649.
73. Weller, J. and Budson, A. 2018. Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Res.* **7**, 1161.
74. Wolk, D. A., Grachev, I. D., Buckley, C., Kazi, H., Grady, M. S., Trojanowski, J. Q., Hamilton, R. H., Sherwin, P., McLain, R. and Arnold, S. E. 2011. Association between in vivo fluorine 18-labeled flutemetamol amyloid positron emission tomography imaging and *in vivo* cerebral cortical histopathology. *Arch. Neurol.* **68**, 1398-1403.

초록 : 말초 아밀로이드 베타 원천으로서의 혈소판과 알츠하이머병의 혈액 바이오마커로서의 가능성

강재선 · 최윤식*
(경성대학교 약학과)

알츠하이머병은 점진적인 신경세포의 손상과 이로 인해 인지기능 장애를 유발하는 질병이다. 이 질환은 현재로서는 치료할 수 있는 질환이 아니고 진행을 멈추게 할 수 있는 방법이 없다. 그러나 초기에 알츠하이머병을 치료하는 것이 가장 효과적이므로 초기 진단은 증상을 관리할 수 있는 가장 좋은 기회를 제공할 수 있다. 알츠하이머병을 진단하기 위한 바이오마커로는 아밀로이드 베타(Aβ), 병적인 타우, 그리고 신경퇴화가 있고, Aβ의 축적, 인산화 타우는 뇌척수액이나 양전자 방출 단층촬영술을 통해 분석할 수 있다. 그러나 뇌척수액의 채취는 매우 침습적이고 양전자 방출 단층촬영술은 전문적인 고가의 장비가 필요하다. 지난 수십년 동안 빠르고 최소한의 침습성을 가진 바이오마커 분석법을 개발하기 위하여 혈액에 기반한 바이오마커 분석 기술이 연구되어 왔다. 그 중 주목할 만 한 발견이 혈장에서 Aβ의 주요 원천으로 혈소판과의 관련성이다. 아밀로이드 베타는 혈액-뇌 장벽을 통과할 수 있고 정상 상태에서는 뇌와 혈액 간 평형을 이루게 된다. 흥미롭게도, 여러 임상시험 결과 혈장에서 $A\beta_{42}/A\beta_{40}$ 비율이 가벼운 인지장애 질환과 알츠하이머병에서 감소되어 있는 것을 증명하였다. 종합하면, 이러한 최근의 발견들은 침습성을 최소화한 알츠하이머병의 초기 진단 기술을 개발하는 데 이용될 수 있다. 본 총설에서, 저자들은 알츠하이머병의 바이오마커에 대한 최근 연구결과들, 특히 말초에서 Aβ를 생산하는 혈소판의 역할과 혈액 기반 바이오마커로서의 개발 가능성에 대해 고찰하였다.