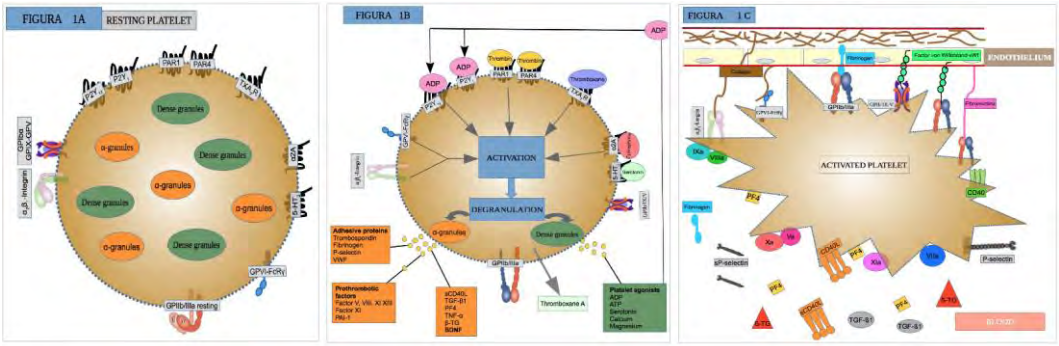
Reviewer's code: 02445328

This is a very detailed review on "peripheral BDNF levels and platelet activation". Although it gets harder for a clinician to follow the whole manuscript, as a investigator in the field I enjoyed reading it. But I am not sure every other investigator would have the appetite as mine. Therefore, I suggest to shorten the manuscript and add few figures for a better understanding.

I introduced 1 figure with 3 imatges to better understanding platelet receptors and the excretion of granule substances. I deleted some paragraphs of the article that were not directly related to the subject



- There are few typo mistakes like in the first sentence in the core tip.

I corrected this part of manuscript and revision with Proof-Reading Service. The first manuscript were revised by American Journal Experts

- Page numbers are missing

I numbered the pages

- Under the section of human studies and chronic antidepressants (it would be better "chronic antidepressant use): the second half of the paragraph is not understood well.

Chronic antidepressant use in humans:

One study evaluated the changes in the platelet BDNF levels in patients with major depression when they were treated with s-citalopram. The platelet BDNF levels of the untreated patients appeared significantly lower than those of the healthy subjects, and

antidepressant treatment with an SSRI normalized the platelet BDNF levels. The platelet BDNF levels were normalized earlier (at eight weeks of treatment) than the plasma BDNF levels[69]. Another study evaluated the platelet BDNF levels in patients with major depressive disorder and childhood trauma. They were treated with antidepressant medications for three months, including escitalopram, mirtazapine, and duloxetine, without intensive psychotherapy. The platelet and serum BDNF levels showed a significant increase from baseline at the 3-month follow-up in the patient group. Conversely, the plasma BDNF levels were not significantly different between the two time points in the patient group. There were no significant differences in BDNF levels between the different antidepressant treatment groups[75] in either of the peripheral samples analysed.

Reviewer's code: 02456929

Title: are the changes in the peripheral BDNF levels due to platelet activation?

The author carried a review in the literature for analyzed studies that evaluated the relationship between BDNF and platelet activation. Very interesting review. In my opinion this could be of interest for the journal and your readers. However the author should modify the manuscript according to the following suggestions:

- General: Consistency between the title of the manuscript, the objectives and the conclusion of his interesting review. I understand that this report not was a systematic report. However, for the reproducibility is recommended that the author gave a date of the last literature searches.

The following review will focus on the relationship between platelet activation and BDNF, particularly in depression. This review will examine current literature regarding this topic until August 2015.

- Title: This could identify the manuscript as a review.

I propose another title: Are the changes in the peripheral bdnf levels due to platelet activation? A review of current knowledge in depression.

批注 [Ed1]: Many journals do not allow abbreviations in the title. Please consult your target journal's Instructions for Authors for specific instructions.

- Conclusion: This section could answer the title question.

To the best of our knowledge, only a few studies have assessed both BDNF and platelet activation in depression[31]. BDNF has been implicated in the pathophysiology of depression[133], and it has been studied as a biomarker of this disease. A large number of clinical studies have reported that the BDNF levels in serum[83,134-136] are significantly decreased in depressed patients and that this decrease is normalized by antidepressant treatments[135,137-140], which was confirmed by meta-analysis[141,142]. Serum levels are influenced by platelets and plasma levels results are inconsistent. Ninety per cent or more of blood BDNF is stored in platelets, but these studies did not consider the platelet alterations observed in depression. Increasing evidence indicates that there is an association between depression and platelet function[152-154], with an elevated platelet reactivity, a prothrombotic endophenotype, increased of plasma substance levels excreted from α-granules in depressed patients. Some authors propose that the lower peripheral BDNF concentrations in depression and their upregulationover the course of antidepressant treatment may be an epiphenomenon resulting from an altered BDNF metabolism or expression by these peripheral organs[3]. One possible explanation could be that alterations in peripheral BDNF levels in depression depend more on platelet reactivity that excretes more BDNF from a-granules than on alterations of central BDNF. A serious question remains to be answered of whether the relationships found between BDNF and depression may be mediated primarily by the relationship between depression and platelet activation. Further studies are required to evaluate the complexity of the relationship between BDNF and platelet reactivity and its possible influence on the peripheral levels in certain diseases, such as depression. Another group of studies is required to evaluate the implications of anti-platelet and antidepressant drugs in the relationship between BDNF and platelet activity.

Reviewer's code: 00504963

This is a very interesting review article addressing the relationship between peripheral BDNF levels and platelet activation. The topic is important because altered levels of blood (serum/plasma)

BDNF have been repeatedly reported in depressive disorder and the role of platelets, which release a substantial proportion of peripheral BDNF, is unclear in the pathophysiology of the illness. The author has made a great effort in the literature search and addressed an adequately wide range of related issues.

Major comments:

1. In the CORRELATIONS BETWEEN THE CEREBRAL AND PERIPHERAL BDNF LEVELS section, the authors wrote that BDNF crosses the blood-brain barrier [92] (L1P14). However, to the reviewer's knowledge, BDNF has not generally been considered to cross the BBB according to Pardridge (Drug Discov Today 12: 54–61, 2007; Pardridge et al Pharm Res 15: 576–582, 1998). These contradictory findings should be both presented and clarified.

Because the central BDNF levels are difficult to obtain for methodological and ethical reasons, there is a great interest in peripheral BDNF measures in relation to psychiatric illness. There are indications that the BDNF measured in peripheral tissues reflects BDNF activity in the brain. These indications include preclinical findings that BDNF crosses the blood-brain barrier[92] and positive correlations between the peripheral and central BDNF concentrations[57,93,94]. Referring to the blood-brain barrier, some rodent studies have shown that peripheral BDNF administration promotes the regeneration of spinal cord injury[95], has an effect on depressive-like behaviour[96] and could increase BDNF levels in the brain[97]. Other studies did not find these results but found that BDNF protein may have poor or null blood-brain barrier penetrability[98-100]. Systemically administered BDNF in rodents showed that BDNF signaling pathways were activated only in disrupted regions[101]. However, studies also point towards a role for vascular endothelial impairment in MDD[102]. A meta-analysis found an increased risk of MDD in those with major vascular diseases including diabetes, cardiovascular disease and stroke[103].

2. The Fibrinogen section (P16) is too thin. Recent studies have reported strong associations between increased fibrinogen levels (blood and CSF) and depression, Wium-Andersen et al Mol Psychiatry 2012;18:854–855 for blood and Hattori et al Sci Rep. 2015; 5: 11412 for CSF, for example. The authors should cite such articles and discuss more about the possible link between, fibrinogen, BDNF, and platelets in the illness.

Fibrinogen:

Similarly to BDNF, fibrinogen is a major storage protein of platelet α-granules and is delivered to the α-granules by endocytosis[113]. Recent studies reported an association between elevated plasma fibrinogen levels and psychological distress and depression in individuals from the general population after adjusting for confounders[114], but the effect size of plasma fibrinogen could be small[115]. Another study found higher fibrinogen levels in non-responders than in responders in major depression patients, suggesting that baseline plasma fibrinogen levels can serve as a biomarker to gauge the success of antidepressant treatment response[116]. Hattori et al found that a subpopulation of patients with MDD had high CSF fibrinogen levels compared with controls and those patients with a high fibrinogen level had white matter tract abnormalities[115]. The increased CSF fibrinogen in patients could represent a trace of blood-brain barrier disruption induced by neuroinflammation, which is in accordance with the mild inflammation hypothesis in the aetiology of MDD[117,118]. The plasma BDNF levels were negatively associated with the fibrinogen levels in patients with angina pectoris[87]. The exact reason for the association between the decreased plasma BDNF levels and these factors is unclear, but it may be related to the level of BDNF release from platelets in inflammatory states.

3. The authors should provide a figure illustrating the possible molecular mechanisms underlying the link between BDNF and other secreted molecules from platelets and depression, which will be of substantial help for readers to understand the article.

I introduced one figure with 3 images to better understanding platelet receptors and excretion from granule substances.

Minor comments

1. In the abstract, the authors describe that secreted BDNF represents 30% of the total BDNF. What is "the total BDNF"? Total BDNF in the peripheral blood or total BDNF in platelets?

Recent studies showed that BDNF is present in two distinct pools in platelets, in α-granules and in the cytoplasm, and only the BDNF in the granules is secreted following protease-activated receptor type 1 (PAR-1) stimulation, representing 30% of the total BDNF in platelets.

批注 [Ed2]: Abbreviations and acronyms are often defined the first time they are used within the abstract and again in the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention. You may wish to consult your target journal to determine which abbreviations are considered common enough that they do not need to be defined.

2. References should be given to the 1st and 2nd sentences of the introduction (concerning 2.5% of the general population and the 2nd highest burden).

Major depressive disorder is a common and invalidating mental illness, with a global point prevalence of 4,7% in general population (Ferrari 2013). Depressive disorder is one of the leading causes of disability, and it has been suggested to become the disease with the second highest burden (Murray 1997- Lancet).

3. The authors should describe for what agonist did they mean? (L16P7)

The binding of biochemical agonists (thrombin, collagen, ADP, epinephrine, arachidonic acid)..

4. L17P9: "CD62P" should be "P-selection (CD 62-p)" to be in accordance with the later description in the "P-selectin" section on page16? The description of the same molecule should be the same throughout the manuscript.

I unified initials into one (CD-62-p)

5. The following sentence in the last paragraph on page 12 is redundant and can be deleted;.Platelets constitute a source of the peripheral BDNF concentrations.

I deleted it.

6. The authors wrote; The BDNF stored in platelets is likely derived from both the circulating plasma pool and from the resident cells in the brain or other organs (L1P14). However, the authors suggest the possibility of pass down from megakaryocytes on page 8. These descriptions are contradictory and confusing.

The BDNF stored in platelets is likely derived from the circulating plasma pool, from cells in the brain or other organs and from megakaryocites.

7. In the beta-TG section (P17), the authors wrote; In contrast, the BDNF and plasma beta-TG values were not significantly correlated. It seems that this sentence should be changed to; In contrast, the plasma BDNF and beta-TG values were not significantly correlated.

I changed it.

8. In the TNF-a section (P18), the descriptions are unclear. In the 1st sentence, how is BDNF

linked to release of proinflammatory cytokines? Does cytokines inhibit or enhance BDNF? In the 2nd sentence, how was serum BDNF significantly associated with serum TNF? Furthermore, the last two sentences of the section seem to be contradictory and confusing.

TNF is a proinflammatory cytokine and is produced by macrophages. It have more relationship with inflmatory process than with coagulation. I think is better to eliminate it of article. In another paragraph I cited the relationship between plasma BDNF and inflammatory response.

9. The description of the 1st paragraph of the "animal studies and single dose of an antidepressant" (P19) is rather confusing. The statement "there were no differences between the effects of serotonin–norepinephrine reuptake inhibitors (SNRIs) and selective serotonin re-uptake inhibitors (SSRIs)" and that of "the changes in BDNF release depend on the type and amount of the antidepressant" seem to be contradictory. The authors should correct these points to be clear.

Antidepressants promoted BDNF release from platelets within 1 hour, and the changes in BDNF release depended on the amount of the antidepressant and they were specific for each antidepressant.

10. Typos / grammatical errors

1) In Core tip: no neuronal -> non-neuronal; Depression disorders -> Depressive disorders .

I corrected this part of manuscript and revision with Proof-Reading Service. The first manuscript were revised by American Journal Experts