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Expression of Tie2 (angiopoietin receptor) on the monocyte subpopulations from ischemic stroke patients: Histological and flowcytometric studies

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Summary. Introduction. Different subpopulations of monocytes play roles in phagocytosis, inflammation, and angiogenic processes e.g., Tie2-expressing monocytes (TEMs). The brain is flooded with macrophages that are derived from monocytes within 3-7 days after a stroke. This study aimed to determine the expression level of Tie2 (an angiopoietin receptor) on monocytes and their subpopulations in ischemic stroke patients using the histological and immunohistological study of bone marrow biopsies and blood flow cytometry examination.

Methods. Ischemic stroke patients within two days were selected. Participants in the control group were healthy volunteers of matched age and gender. Sample collection was performed within 24 to 48 hours after medical consultants confirmed the stroke diagnosis. An iliac crest bone marrow biopsy was obtained and fixed for histological and immunohistological staining with antiCD14 and antiCD68. Flow cytometry was used to determine the total monocyte population, monocyte subpopulations, and TEMs after staining with monoclonal antibodies to CD45, CD14, CD16, and Tie2.

Results. Post-stroke patients' bone marrow cells were hypercellular. There was an apparent increase in CD68 and CD14-positive cells. Ischemic stroke patients exhibited low percentages of nonclassical monocytes CD14lowCD16++, with an increase in intermediate monocytes CD14highCD16+. Moreover, ischemic stroke patients had significantly higher levels of TEMs than control group.

Conclusions. The results of this study demonstrate dysregulation of angiogenesis in monocyte subsets in ischemic stroke patients, which could be used as an early diagnostic marker of neurovascular damage and may need angiogenic therapy or improved medications to prevent further damage of blood vessels.

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Introduction

Stroke is among the top three leading causes of disability and mortality in the world. There are approximately 16.9 million strokes worldwide each year, according to World Health Organization. By the year 2030, a significant number of stroke deaths and cases of disability will be reported around the world (Owolabi et al., 2021). Ischemic strokes are more common than hemorrhagic strokes. About 80% of stroke cases are caused by ischemia worldwide (Feigin et al., 2014; Katsoularis et al., 2021).

According to Saudi Arabian statistics, 22% of deaths caused by cardiovascular diseases (Alrafiah et al., 2021). In Saudi Arabia, stroke cases estimated to occur at a rate of 29.8 out of 100,000 (Alahmari and Paul, 2016). People with diabetes mellitus (DM) have a fourfold higher stroke risk (Tun et al., 2017). In a country like Saudi Arabia, where DM has been a major epidemic for the last three decades, stoke is one of the most serious complications (Al Dawish et al., 2016).

Due to the high mortality and disability associated with strokes, new therapeutic and diagnostic approaches are urgently needed. The blood-brain barrier (BBB) is hypothesized to be damaged by an acute neurovascular injury that leads to ischemic stroke and death of neural cells. As part of stroke pathogenesis, delayed neurovascular repair (angiogenesis, neurogenesis) is also involved (Al-ofi et al., 2012). In addition to their function as phagocytic subsets, different monocyte types also serve as pro-inflammatory and pro-angiogenic subtypes as Tie2-expressing monocytes (TEMs) (Strauss-Ayali et al., 2007). Stroke patients had angiogenesis and neuro-angiogenesis. However, Tie2 and monocyte subsets must play a significant part in developing angiogenesis and treating stroke patients.

During microglial activation after ischemic stroke,

cell morphology is changed either to M1 typically activated phenotype, or to M2, an alternatively activated phenotype; this phenotypic switch depends on type of stimulation. M2 microglia are regarded as "healing cells" that led to recovery after damage and secrete anti-inflammatory mediators like transforming growth factor-β (TGF-β), interleukin- (IL-) 10, IL-13, IL-4, and insulin growth factor- (IGF-) 1, as well as various neurotrophic factors (Ponomarev et al., 2013; Taylor and Sansing, 2013). M1 microglia formed proinflammatory meditators like tumor necrosis factor- α (TNF- α), IL-β, and interferon- γ (IFN-γ). M1 microglia express CD86, CD80, and MHC class II on cell membrane and present antigens to T cells (Starossom et al., 2012). M1 microglia induced neuronal cell death more readily than M2 microglia (Hu et al., 2012).

Various immune cells release cytokines, chemokines, and angiogenic molecules to counteract the ischemic stroke effects. Potential biomarkers can be developed using these molecules to diagnose and monitor ischemic strokes. There are several markers of ischemic stroke in patients, including Tie2 (angiopoietin receptor) expression in monocytes and subpopulations of monocytes. Bone marrow biopsies and blood flow cytometry were used to assess levels of Tie2 (angiopoietin receptor) expression on monocytes and their subpopulations in patients with ischemic stroke. This study aimed to determine the expression level of Tie2 (an angiopoietin receptor) on monocytes and their subpopulations in ischemic stroke patients using the histological and immunohistological study of bone marrow biopsies and blood flow cytometry examination.

Materials and methods

Study population

The study was conducted at King Abdulaziz university hospital, Jeddah, Saudi Arabia, under ethical approval number No 53217. This study enrolled the healthy control group (n=10) and the ischemic stroke patient group (n=12). Patients with active infections or inflammation were excluded. Samples were collected in the morning within 24 to 48 hours after medical consultants confirmed stroke diagnosis. The demographic data of the included subjects are listed in Table 1. In addition, patients with ischemic stroke were matched with controls for age and gender.

Biopsies and tissue preparation

Bone marrow trephines were used for biopsies. The core specimens were fixed in 10% Phosphate Buffered Formalin solution (PBF), then processed using an automated tissue method. A rotary microtome was used to cut paraffin blocks into slices 3 to 5 mm thick. Slices were incubated on a glass slide for 24 hours in a warm water bath set at 40°C. After cooling, slices were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Serial paraffin slices were stained with two markers for monocyte identification during immuno-histochemical staining. After slides were fixed, deparaffinized, and hydrated, endogenous peroxidases were inhibited by 0.5% hydrogen peroxide in methanol treatment for 10 minutes. For antigen retrieval, slices were microwaved twice for about 2 minutes each after incubating in 10 mM citrate buffer with pH6.5. After cooling, sections received a standard saline treatment after 5-minute washing in PBS (pH 7.6). For two hours, sections were exposed to anti-CD14 mouse primary antibodies (Novocastra, France). Biotinylated goat antirabbit and antimouse antibodies (ABCys, Paris, France) were used to identify the binding of anti-CD14 mouse antibodies (ABCys, Paris, France).

Utilizing diaminobenzidine, peroxidase activity was created (DakoCytomation, Trappes, France). Other regions received anti-CD68 (PG-M1) mouse antibody treatment for an hour (DAKO, Denmark). After using a rabbit antimouse antibody to identify anti-CD68 antibody binding, 5-minute incubation with a 1 mg/ml DAB solution was performed (3,3'-tetrahydrochloride diaminobenzidine, Sigma, USA). Monoclonal antibody against CD68 was used to label macrophages produced from monocytes. Before being mounted in DPX, slides were counterstained with Mayer's hematoxylin. Then, each piece was carefully scanned, analyzed, and captured on camera.

Blood samples

Fresh blood samples (5 ml) were collected from participants in Ethylenediaminetetraacetic acid (EDTA) tubes (Becton Dickinson, San Jose, USA). A Sysmex XS whole blood analyzer was used to perform whole blood counts (Sysmex Corporation, Kobe, Japan).

Immunophenotyping

Immunophenotyping tests were conducted on 12 newly diagnosed ischemic stroke patients. The US Company Bio Legend offered fluorescent CD markers. Selected antibodies conjugated to these markers along with allophycocyanin (APC), peridinin chlorophyll

Table 1. Demographics and clinical criteria of ischemic stroke patients (ISP) and controls.

	Control (n=10)	Ischemic stroke patients (n=12)
Age (years)	55 (44-60)	55 (44-65)
Male	6 (60.00%)	7 (58.33%)
Female	4 (40.00%)	5 (41.67%)
Positive smoking history	-	3 (25.00%)
Hypertension	-	6 (50.00%)
Type 2 diabetes mellitus	-	3 (25.00%)

protein (PerCP), fluorescein isothiocyanate (FITC), and phycoerythrin (PE). In addition, leukocyte immunophenotyping (surface staining) was carried out using FACS CantoTM II and CD16-FITC (monocytes), CD14-PerCP/Cy5.5 (monocytes), CD202b (Tie2), and CD45-APC (leukocytes) (Table 2).

Flowcytometer calibration

The cytometer was set up using BD FACS Diva CS&T beads, which the software used to describe, track, and report cytometer measurements. Daily performance quality controls (QC) and lyse/wash (LW) application parameters were determined using these beads.

Software algorithms calculated the cytometer baseline. After establishing baseline mean fluorescence intensity (MFI) objective values, beads were used to perform daily performance assessments. BD FACS Diva CS&T IVD beads were used to reset MFI target values when switching to a fresh batch of beads. Additionally, BD FACS Diva CS & T IVD beads were used to establish LW application parameters manually.

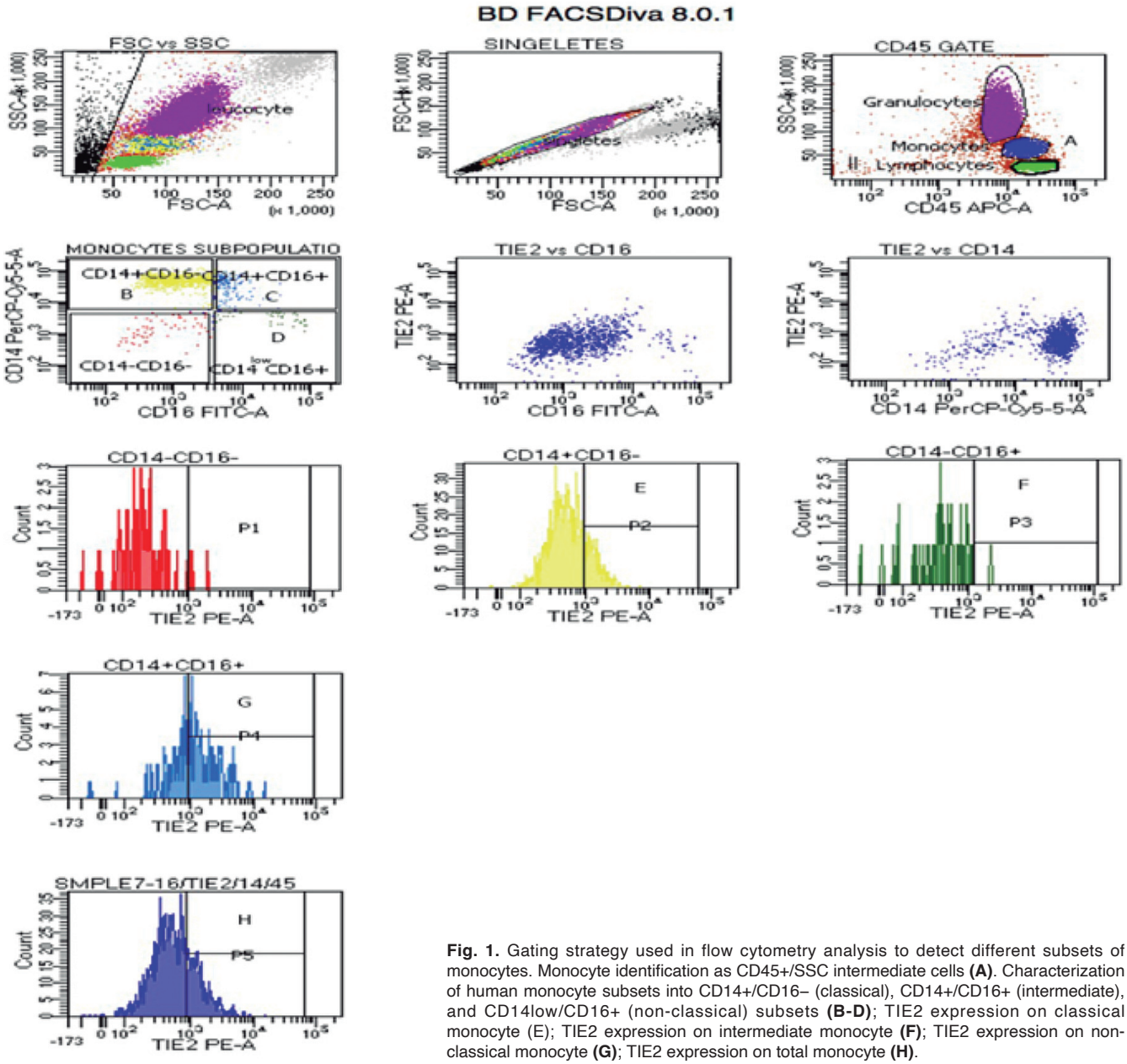

Fig. 1. Gating strategy used in flow cytometry analysis to detect different subsets of monocytes. Monocyte identification as CD45+/SSC intermediate cells **(A)**. Characterization of human monocyte subsets into CD14+/CD16– (classical), CD14+/CD16+ (intermediate), and CD14low/CD16+ (non-classical) subsets **(B-D)**; TIE2 expression on classical monocyte (E); TIE2 expression on intermediate monocyte **(F)**; TIE2 expression on non-classical monocyte **(G)**; TIE2 expression on total monocyte **(H)**.

Following the user's dieter performance assessment, the program parameters were entered.

Fluorescence compensation

BD™ Comp Beads Set Anti-Mouse Ig, κ are polystyrene microparticles that were used to optimize fluorescence compensation settings for multicolor flow cytometric analyses. The set provides two populations of microparticles, BD™ Comp Beads Anti-Mouse Ig, κ particles, which bind any mouse κ light chain-bearing immunoglobulin, and BD™ Comp Beads Negative Control, which has no binding capacity. Four tubes, each containing 100 µl of staining buffer BSA stained with CD16-FITC, CD202-PE, CD14-PerCP, and CD45-APC (positive control) and one tube containing $1x10^6$ white blood cells (WBCs) were subjected to sample processing, with equivalent volumes of PBS in place of CD markers (unstained cells) for electronic optimization. One drop of BD™ Comp Beads Anti-Mouse Igκ was added to positive control tubes, and one drop of Comp Beads Negative Control was added to the unstained tube (negative control). All incubations were performed at ambient temperature unless otherwise stated-cells stained with CD markers for 15 minutes in the dark. The tubes were then incubated in 2 mL FACS Lyse solution (BD Biosciences, San Jose, U.S.A) for 10 min in the dark. Cells were centrifuged at 300xg for 5 min, washed in 2 mL cell wash, and re-centrifuged at 300xg for 3 min.

Forward and side scatter optimization

CD45, forward scatter (FSC), amplification (amp) gain, and CD45 adjusted for the three major leukocyte subpopulations, lymphocytes, monocytes, and granulocytes were used.. A change was made to the FSC threshold to reduce trash accumulation. All samples were optimized for FSC and CD45 under all conditions. Lymphocytes and monocytes were gated using a conjugated CD45 vs. SSC plot. To facilitate the gating of leukocyte types (granulocytes, monocytes, and lymphocytes), $1.0x10^6$ cells stained with CD45-PerCP or CD45-PE-CyTM5 were used. BD FACS CantoTM II (CA, USA) was used to collect and analyze the data. For each sample, 20,000 events were collected.

Expression of surface antigens on monocyte subsets

In order to analyze surface antigens, three BD Falcon FACS tubes were used, one for the negative control, the second for all antibodies, and the third for the internal control. The three BD Falcon tubes contained (100 ml) of whole blood and CD45. CD14 and CD16 were added to tubes 2 and 3. Only Tube 2 received CD202. All tubes were incubated for 15 minutes in the dark. Then, 2 ml of BD FACS lysing solution was added to each tube and incubated for 10 minutes. After that, cell wash PBS was used twice to wash the tubes. Next, 500 µL was added to each tube before being placed into FACS Canto™ II Flow

Table 2. Antibodies used for flowcytometry.

Materials	Catalogue #	Supplier
(PE) anti-human CD202b (Tie2) Antibody	334206	BioLegend (San Diego, USA)
(PerCP/Cy5.5) anti-human CD14 Antihody	367110	BioLegend (San Diego, USA)
(FITC) anti-human CD16 Antibody	360716	BioLegend (San Diego, USA)
(APC) anti-human CD45 Antibody	20021658	Dako (CA, USA)

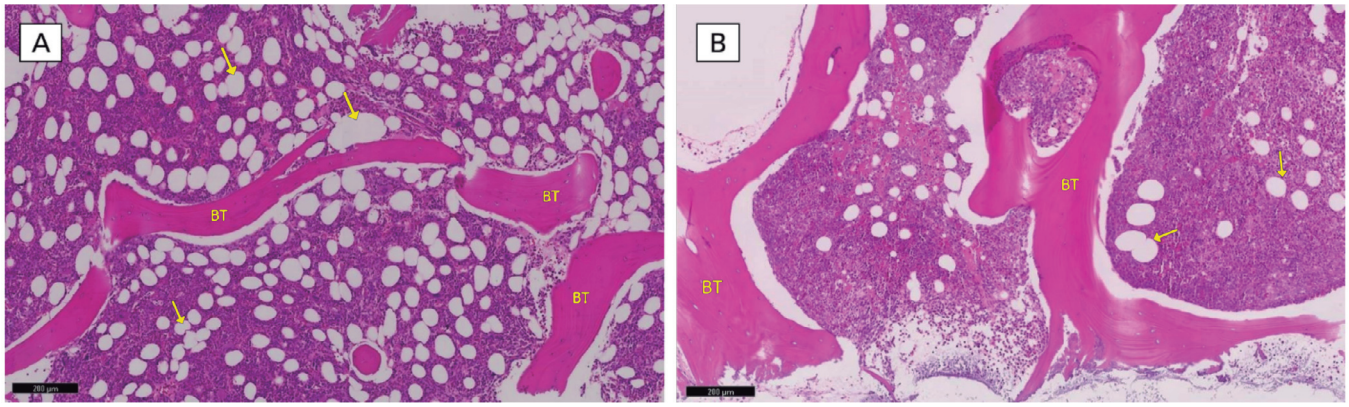

Fig. 2. Photomicrographs of the H&E section of the bone marrow trephine biopsy. **A.** Control subject with bone trabeculae (BT) radiating between bone marrow cells, blood sinusoids and adipose tissue (arrows). **B.** Ischemic stroke patient case has hypercellular marrow between bone trabeculae (BT) with an apparent decrease in adipose tissue (**arrows**). Scale bar: 200 μm

Cytometer.

Monocytes were divided into three primary categories based on CD14 and CD16: classical $CD14^{high}CD16^{-}$, non-classical $CD14^{low}CD16^{+}$, and intermediate $CD14^{+}CD16^{+}$ (Fig. 1).

Statistical Analysis

The Unpaired student's T-test was used to compare mean difference. Pearson test was used to calculate the correlation coefficient. Analysis was made using GraphPad Prism version 8.00 for Mac (GraphPad Software, La Jolla, California, USA, www.graphpad.com). $P<0.05$ level was considered significant.

Results

Histology Results

The typical human bone marrow structure, as shown in the H&E sections from the trephine biopsy of the control participants' bone marrow, includes various clusters of red blood cells (RBCs), WBCs series, and megakaryocytes in between the blood sinusoids. In addition, acidophilic bone trabeculae were seen between the bone marrow tissues (Fig. 2A). The existence of the erythroid series, granulocyte series, and megakaryocytes may be seen at greater magnification in the majority of the sections that have been analyzed (Fig. 2A). The patient had hypercellular bone marrow between bone trabeculae and an apparent reduction in adipose tissue, according to an examination of H&E sections from the trephine biopsy of the bone marrow (Fig. 2B).

Most of the sections that were studied at a greater magnification showed an apparent rise in the granulocyte series at the cost of the erythroid series. The bone marrow cells and dilated blood sinusoids made it easy to see the megakaryocytes (Fig. 3B).

The sparse distribution of brown-stained CD14 positive monocytes in the control participants' CD14 immunostained sections was observed (Fig. 4A). Compared to the studied control sections, the CD14 immunostained sections from the ischemic stroke patients showed an evident increase in the clusters of the CD14 brown stained immunopositive cells (Fig. 4B). The control participants' CD68 immunostained sections showed a sparse amount of brown-stained CD68 positive cells (Fig. 5A). Compared to the studied control sections, the CD68 immunostained sections from the ischemic stroke patients demonstrated a noticeable increase in the CD68 brown-stained immunopositive cells (Fig. 5B).

Flow cytometric results

Compared to the control group, ischemic stroke patients had substantially more total leukocytes ($P<0.05$) (Fig. 6a). Furthermore, compared to the control group, the total number of monocytes was considerably more significant in the case group ($P<0.001$) (Fig. 6b).

The monocyte subpopulations, however, varied significantly across the three groups. In contrast to the control group, ischemic stroke patients showed a significantly reduced percentage of non-classical $CD14^{low}CD16^{+}$ monocytes ($P<0.05$) but a very significantly higher proportion of intermediate monocytes $CD14^{+}CD16^{+}$ (Fig. 7a,b). Classical monocytes with CD14highCD16-, however, did not significantly vary between patients and controls ($P=0.489$) (Fig. 7c).

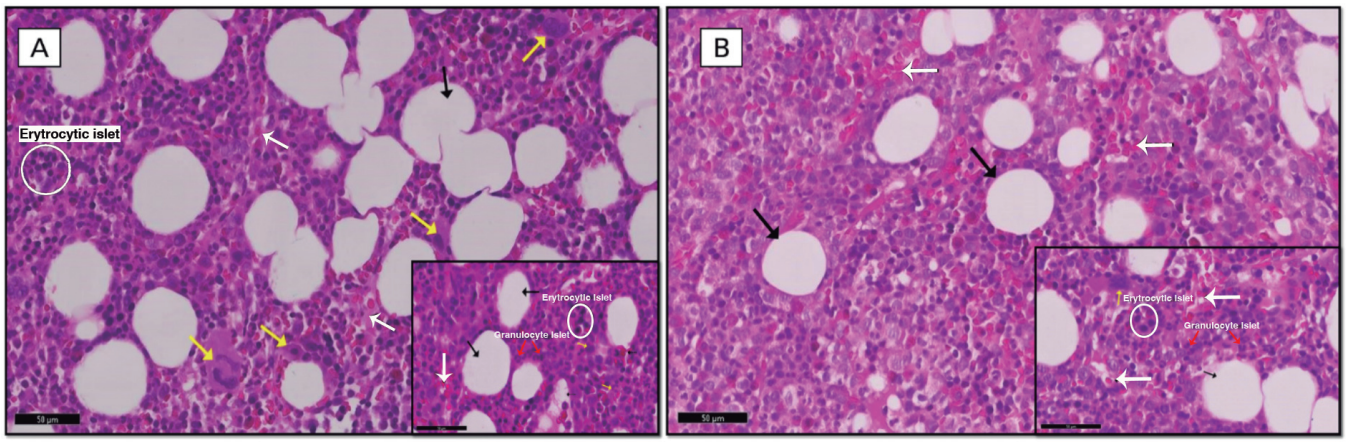

Fig. 3. Photomicrographs from the H&E section from the bone marrow trephine biopsy. **A**. Acontrol subject, with an apparent increase of the adipose tissue (**black arrows**) and in-between blood sinusoid (white arrows), compared to the cellular elements. **Inset:** higher magnification showing the distribution of the erythroid series, granulocyte series (red arrow), and megakaryocytes (yellow arrow). **B.** An ischemic stroke patient with hypercellular marrow with an apparent decrease of the adipose tissue (**black arrows**) and an apparent increase of dilated blood sinusoids (white arrow). **Inset:** higher magnification showing erythroid series and an apparent increase of granulocyte series (red arrow) with megakaryocytes (yellow arrow). Scale bar: 50 μm

Percentage of TEMs in peripheral blood monocytes in different groups

Compared to the control, ischemic stroke patients showed a substantial increase in monocytes expressing TIE2. Using flow cytometry, the expression of Tie2 was evaluated in circulating monocytes from ischemic stroke patients and matched controls. When ischemic stroke patients were compared to the control group, the level of circulating TEMs was considerably greater ($P<0.001$) (Fig. 8a). Additionally, stroke patients had a significant increase in the expression of Tie2 on Intermediate monocytes (pro-angiogenic phenotype) compared to the control group ($P<0.001$) (Fig. 8.b). Furthermore, when ischemic stroke patients were compared to the control, there was a highly significant increase in the Tie2 on non-classical monocytes (proinflammatory phenotype) ($P<0.001$) (Fig. 8c). Finally, when ischemic stroke patients were compared to control people, there was a highly significant increase in the Tie2 on Classical Monocytes ($P<0.001$) (Fig. 8d).

There was a strong positive association between the percentage of Tie2 on classical monocytes and the percentage of Tie-2 on total monocytes when stroke patients' peripheral blood monocytes were analyzed ($r=0.622$; $P<0.05$) (Fig. 9).

Discussion

A stroke influenced by adaptive immunity because monocytes are primary immune cells (Karikó et al., 2004). In addition to their ability to differentiate into

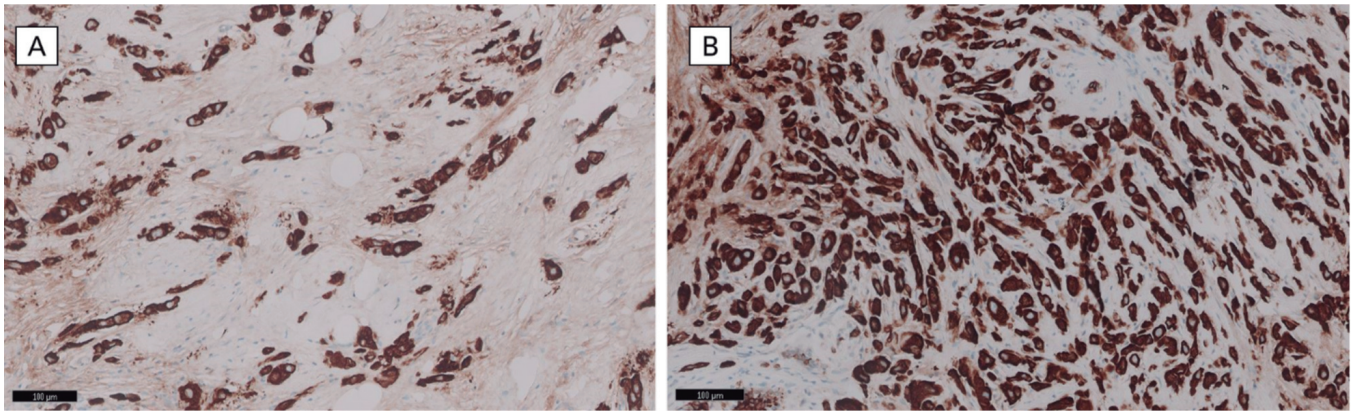

Fig. 4. Photomicrographs from sections of the bone marrow trephine biopsy immunostained by CD14. **A.** Sparse loose clusters of the CD14 brown stained immunopositive cells in a control patient. **B.** An apparent increase in the clusters of the CD14 brown stained immunopositive cells in an ischemic stroke patient. Scale bar: 100 μm.

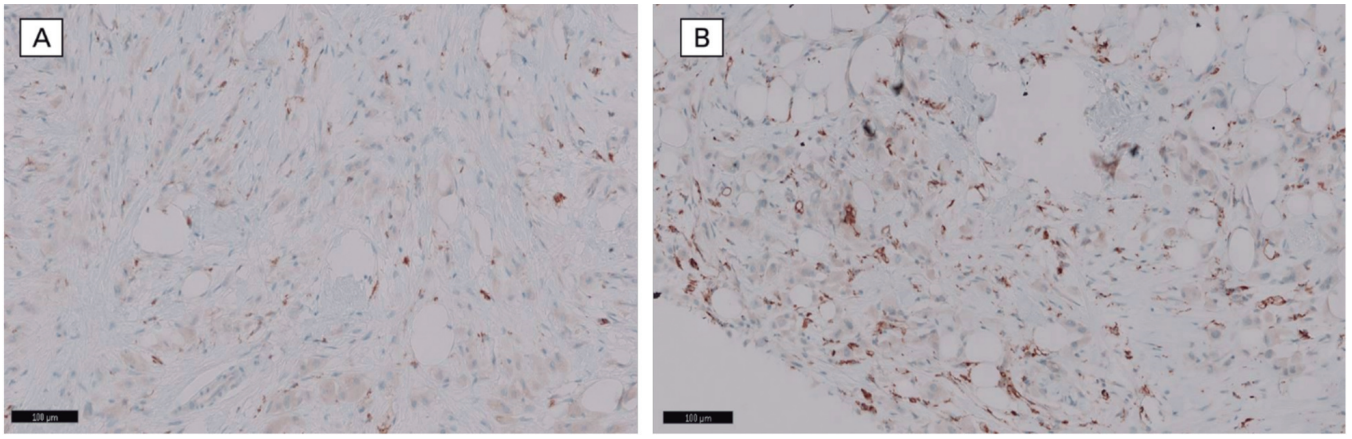

Fig. 5. Photomicrographs from sections of the bone marrow trephine biopsy immunostained by CD68. **A.** A control subject has a sparse number of the brown stained CD68 immunopositive cells. **B.** An ischemic stroke patient section shows an apparent increase in the brown stained CD68 immunopositive cells. Scale bar: 100 μm.

macrophages and dendritic cells, monocytes also participate in initiating the immune response in the presence of infections in which heat-shock proteins, RNA, fibronectin, or fibrin are generated (Dénes et al., 2005). As reported in the current study, bone marrow sections stained with Haematoxilin-Eosin stain revealed hypercellularity in ischemic stroke patients. A similar picture observed by some investigators (Denes et al., 2011; Yang et al., 2012; Courties et al., 2015). Courties et al. hypothesized that after a brain injury, such as a stroke, mononuclear nuclear bone marrow cells may differ in number and quality from mononuclear nuclear bone marrow cells of healthy animals. They compared mononuclear cells produced by the same animals before and after a stroke (Courties et al., 2015). In another study, it was found that strokes' influence on leukocyte response fluencies had been linked to the central nervous system's autonomic regulation of bone marrow (Yang et

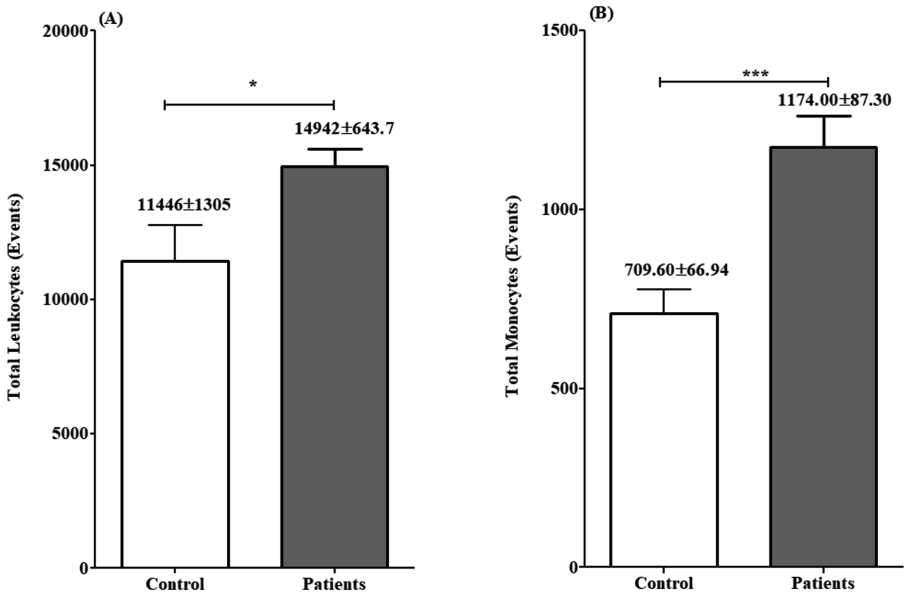

Fig. 6. Comparison between patients and control of total leukocytes (events) level **(A)** and total monocytes (events) level **(B)**. Data are expressed as mean ± standard error.

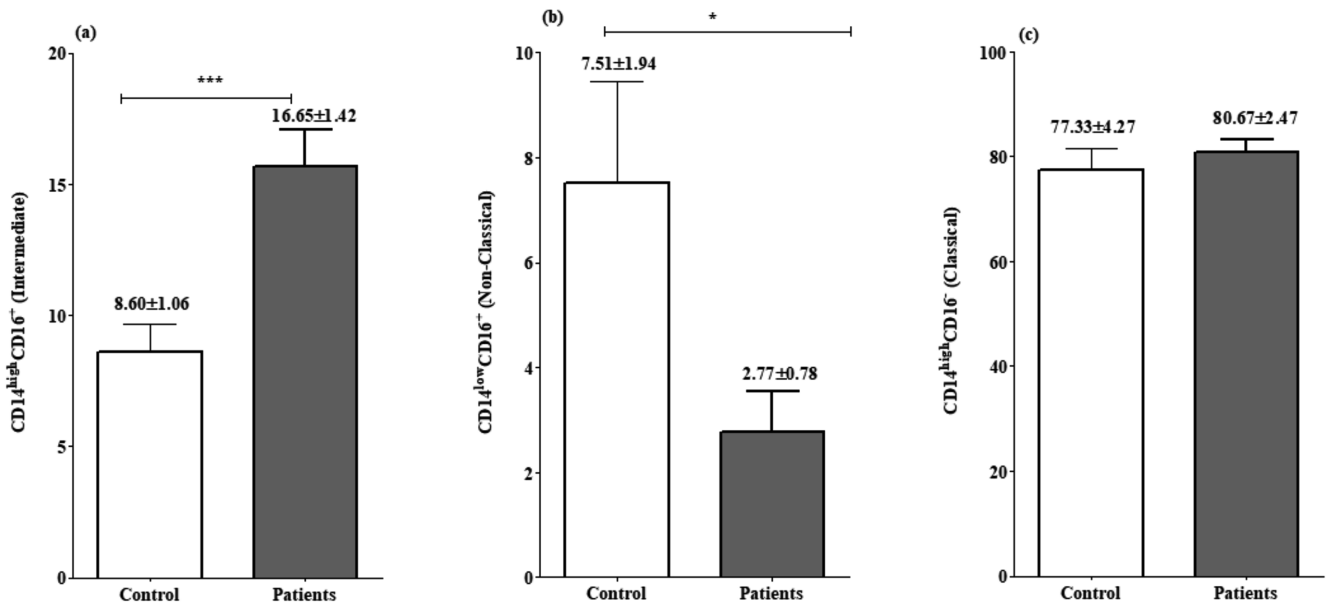

Fig. 7. Comparison between patients and control. **a.** $CD14^{high}CD16^{+}$ (intermediate) level. **b.** $CD14^{low}CD16^{+}$ (non classical). **c.** $CD14^{high}CD16^{+}$ (classical) level. Data are expressed as mean ± standard error.

al., 2012).

The circulation of myeloid cells is higher after stroke, especially neutrophils and monocytes. The proliferation signals are also increased as well. In animals with strokes, the hematopoietic progenitor cell activity led to an increase in the colony numbers after seven days (Courties et al., 2014; Ginhoux and Jung, 2014).

It has been found that monocytes exhibit several clusters of differentiation, including CD115, CD11c, CD14 and CD16 in humans and CD115, CD11b, and Ly6C in rodents (ElAli and Jean LeBlanc, 2016). As compared to control group, ischemic stroke patients appeared to have an increase in CD14 immunostained positive cells in the current study. Monocytes in humans can be classified into three main subsets based on their CD14 and CD16 expression levels: classical subsets ($CD14^{++}CD16^{*}$), intermediate subsets ($CD14^{++}CD16^{+}$) and non-classical subsets ($CD14^{+}CD16^{++}$) (Yeo et al., 2019).

Microglia/macrophages are marked by CD68, a lysosomal glycoprotein that shuttles between endosomes, lysosomes, and the plasma membrane in vesicles (Brettschneider et al., 2012). In addition to our findings, several studies (Schilling et al., 2009; Hendrickx et al., 2017) had also found an increase in CD68 positive staining in bone marrow cells. Blood-derived macrophages are abundantly expressed 3-7 days after middle cerebral artery occlusion, even though they were hard to distinguish from CD68-positive cells (Urra

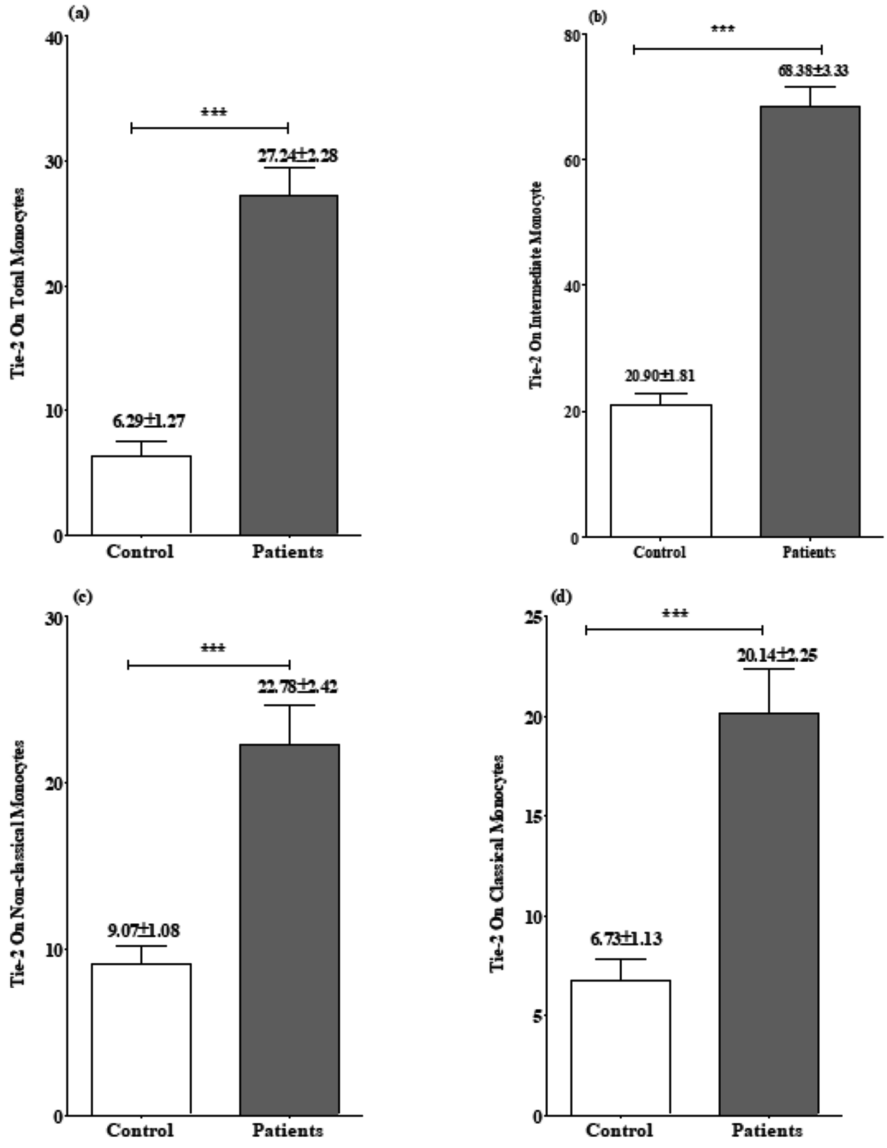

Fig. 8. Comparison between patients and control **a.** Tie-2 on total monocytes level. **b.** Tie-2 on intermediate monocyte level. **c.** Tie-2 on non-classical monocytes level. **d.** Tie-2 on classical monocytes level. Data are expressed as mean ± standard error.

et al., 2009).

The current data showed that compared to control, the percentages of intermediate monocytes increased, whereas non-classical monocytes declined and classical monocytes remained unchanged. In agreementwith Kaito et al., a monoclonal antibody used in that phenotyping study enhanced the $CD14^{high}CD16^{-}$ classical monocyte subtype (Kaito et al., 2013). Our results agree with others (Kaito et al., 2013; Burbano et al., 2014).

Compared with other monocyte subsets, $CD14^{low}CD16^{+}$ non-classical monocytes had lower Tie2 expression in ischemic stroke patients. Patients with atherosclerosis, rheumatoid arthritis, sepsis, HIV infection, and systemic lupus erythromatosis have high levels of non-classical monocytes (Kawanaka et al., 2002; Auffray et al., 2009; Ziegler-Heitbrock et al., 2010; Dutertre et al., 2012; Burbano et al., 2014; Ziegler-Heitbrock, 2015). As a result, non-classical monocyte growth might be typical in inflammatory and infectious disorders. However, their involvement in the appearance or spread of these illnesses is unknown (Brown et al., 2000; Auffray et al., 2009).

The current study's fundamental discovery is that various monocyte subpopulations from ischemic stroke patients had dysregulated Tie2 expression levels compared to control participants (De Palma et al., 2005; Dalton et al., 2014). There was also a skewed distribution of monocyte subsets in stroke patients, with angiogenic (TEM) subsets outnumbering inflammatory (Capoccia et al., 2008). TEMs are proangiogenic myeloid cells that circulate and infiltrate tumors (He et al., 2015; Abbott et al., 2017). The findings suggest that Tie2 expressing monocytes are essential in creating tumor blood vessels, and they also point to a potential target for controlling tumor angiogenesis and proliferation (He et al., 2015; Abbott et al., 2017).

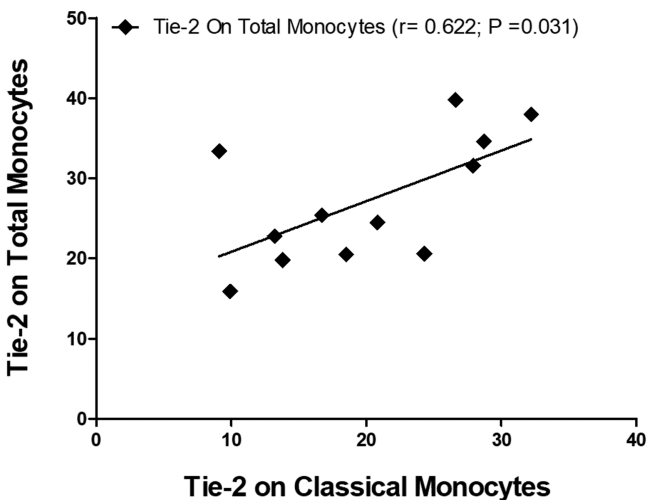

Fig. 9. Correlations coefficient between Tie-2 On Classical Monocytes and Tie-2 On Total Monocytes in patients (r=0.622; P<0.05) using Pearson test.

Although the frequency of TEMs in patients with CLI is more than 10-fold higher than in matched controls, the study showed that the muscle variation, while substantial, is less established. Poor limb perfusion caused by critical ischemia may reduce TEM recruitment to ischemic limb in CLI patients. Despite elevated levels of circulating angiogenic factors, poor limb perfusion is thought to be responsible for the absence of muscle revascularization (Patel et al., 2013).

Additionally, the recruited TEMs are unlikely to survive in the unreceptive milieu of an ischemic muscle after recruitment. It is crucial to realize that TEMs members have increased circulation only because vital ischemia exists, not because it is severe. Moreover, there were no common clinical signs linked to TEM circulation were shown (Amo et al., 2004; Barton et al., 2005; Patel et al., 2013).

According to the findings, TEMs split into two subsets: $CD16^{+}$ monocytes and intermediate CD14++ $CD16^{+}$ cells, depending on strength of CD14++ CD16+ expression. As a result, intermediate monocyte subset had been shown to differentiate between TIE2 high levels and other proangiogenic genes such as VEGFR2 and endoglin (Fernandez Pujol et al., 2000; Ruan et al., 2015).

Future functional investigations should be designed using an *in vitro* angiogenesis test with a tube formation assay. The researchers will look at how monocytes from stroke patients affect brain endothelial cell angiogenesis and whether Tie2 mediates this.

Limitations of the study

One of the study's limitations is the small number of ischemic stroke patients. Including only ischemic stroke is another limitation, as other types of stroke may be studied. The effect of therapy on the expression of Tie2 was also not studied in this study.

Conclusion and recommendations

The evidence gathered in this study showed that monocyte subsets' angiogenic activity is dysregulated in ischemic stroke patients. There was an apparent increase in CD68 and CD14 positive cells and an increased in intermediate monocytes $CD14^{high}CD16^{+}$, while decreased percentages of non-classical monocytes $CD14^{low}CD16^{++}$. Moreover, ischemic stroke patients had significantly higher levels of TEMs than controls. Their discovery in ischemic stroke patients might serve as an early diagnostic signal for initiating neurovascular repair treatment or medication to improve blood vessel status and prevent further damage. In addition, the new findings serve as a new foundation for rethinking how we think about global post-ischemic inflammation. The monocyte subgroup is differential and temporal severity dependent. These stroke pathobiology-related regulations may enable the creation of more innovative and effective therapy techniques.

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Institutional Review Board Statement. The study was designed with correspondence to the Ethics Committee of Biomedical Research-Faculty of Medicine at King Abdulaziz University, ethical approval number (Reference No. 237-20, 31 May 2020). The study was executed in consensus with the guidelines followed in King Fahd Center for Medical Research, KAU, Jeddah, Saudi Arabia, which were in accordance with the principles of the declaration of Helsinki.

Informed Consent Statement. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement. The data used to support the findings of this study were included within the article.

Conflicts of Interest. The author declares no competing interests.

Author Contribution. A.A.: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Visualization, Investigation, Supervision, Software, Validation, Writing- Reviewing and Editing.

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