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Journal

Seminars in Immunopathology, 45(1)

ISSN

1863-2297

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Publication Date

2023

DOI

10.1007/s00281-023-00984-6

Peer reviewed

HHS Public Access

Author manuscript

Semin Immunopathol. Author manuscript; available in PMC 2023 March 20.

Published in final edited form as:

Semin Immunopathol. 2023 January ; 45(1): 125–143. doi:10.1007/s00281-023-00984-6.

Neuroimaging is the new "spatial omic": multi-omic approaches to neuro-inflammation and immuno-thrombosis in acute ischemic stroke

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Abstract

Ischemic stroke (IS) is the leading cause of acquired disability and the second leading cause of dementia and mortality. Current treatments for IS are primarily focused on revascularization

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of the occluded artery. However, only 10% of patients are eligible for revascularization and 50% of revascularized patients remain disabled at 3 months. Accumulating evidence highlight the prognostic significance of the neuro- and thrombo-inflammatory response after IS. However, several randomized trials of promising immunosuppressive or immunomodulatory drugs failed to show positive results. Insufficient understanding of inter-patient variability in the cellular, functional, and spatial organization of the inflammatory response to IS likely contributed to the failure to translate preclinical findings into successful clinical trials. The inflammatory response to IS involves complex interactions between neuronal, glial, and immune cell subsets across multiple immunological compartments, including the blood-brain barrier, the meningeal lymphatic vessels, the choroid plexus, and the skull bone marrow. Here, we review the neuro- and thrombo-inflammatory responses to IS. We discuss how clinical imaging and single-cell omic technologies have refined our understanding of the spatial organization of pathobiological processes driving clinical outcomes in patients with an IS. We also introduce recent developments in machine learning statistical methods for the integration of multi-omic data (biological and radiological) to identify patient-specific inflammatory states predictive of IS clinical outcomes.

Keywords

Stroke; Disability; Hemorrhage; Inflammation; Immunology; Neuroimaging; Single-cell analysis

Introduction

Stroke is the leading cause of acquired disability and the second leading cause of dementia and mortality in the world [1]. According to the *World Stroke Organization*, there are over 12.2 million new strokes each year, and globally one in four people over 25 years will have a stroke in their lifetime, 53% of them occurring in women [1]. Importantly, 67% of people who have experienced a stroke and are currently alive are under 70 years of age, of which 22% are between 15 and 49 years of age [1]. Annually, 6.5 million people die from strokes and over 143 million years-of-life-lost are due to stroke-related death or disability [1], underscoring the tremendous public health burden caused by stroke on a global scale. The most common entity among strokes is acute ischemic stroke (AIS), which accounts for approximatively 80% of all strokes, with 7.6 million new AIS each year worldwide and 77 million people currently living with a history of AIS [1]. AIS is the result of an acute cervical or intracranial arterial occlusion, leading to a profound reduction in cerebral blood flow that causes necrosis of the downstream parenchyma. The mainstay of AIS treatment is prompt revascularization of the occluded artery (i.e., *time is brain*). Revascularization remains the main prognostic factor and can be achieved pharmacologically using intravenous thrombolysis (IVT) or, since 2015, mechanically by endovascular therapy (EVT) when the AIS is the consequence of a large arterial occlusion [2]. EVT has revolutionized the management of AIS due to a large vessel occlusion, with successful reperfusion rates now exceeding 90% [2]. However, due to extremely restricted time frames, less than 10% of patients are eligible for revascularization therapies [3], and only half of successfully treated patients with EVT reach functional independence at 3 months [2]. Altogether, this dissatisfying situation has raised great interest in the

development of new and innovative strategies, in addition to revascularization, to improve the overall prognosis.

Over the past few years, several seminal works have shed light on the deleterious impact of sterile inflammation, which takes place in the short-, mid-, and long-term after AIS [3–5]. This inflammation is strongly associated with overall prognosis (disability, mortality, and post-stroke cognitive impairments), and follows a strict chronology with the involvement of different cells of the innate and adaptive immune system [4, 5]. From a spatial and anatomical perspective, the infiltration of peripheral myeloid and lymphoid cells into the brain parenchyma and astrocyte and microglia, the brain's resident immune cell, response to AIS are commonly referred as the neuro-inflammatory response, which has been described in strokes and other neurological disorders (traumatic brain injuries, multiple sclerosis, encephalitis) [4]. Interestingly, several works have also described an acute inflammatory response within the macrovascular and microvascular circulation, leading to thrombosis and downstream microcirculatory perfusion impairments, also called immuno-thrombosis or thrombo-inflammation [6]. Recent clinical findings have highlighted the prognostic value of thrombo-inflammation, which may result in futile recanalization without reperfusion, also known as the *no-reflow phenomenon*, due to platelet and neutrophils aggregates in the microcirculation [6]. As described below, the neuro- and thrombo-inflammatory pathways are in constant interplay but may recruit immune cells through different compartments within the central nervous system (CNS). These findings led to the development of several experimental treatments or the assessment of anti-inflammatory or immuno-modulatory drugs already in use in the field of clinical neuro-immunology (i.e., multiple sclerosis and other auto-immune diseases), with the purpose of dampening the immune response after AIS, but altogether with conflicting or disappointing results [7]. Among several likely explanations for these results, recent data have refined our knowledge regarding the organization of the immune system within the CNS and, more specifically, the relevance of its spatial organization within the parenchyma, the meninges, the choroid plexus (ChP), or more recently the skull bone marrow, in physiology or disease [8–10]. Second, the development of new technologies, such as mass cytometry (CyTOF) or single-cell RNA sequencing (scRNAseq), provided new insights into the cellular activation state at the proteomic and transcriptomic level, challenging the overly-simplified view previously held regarding the dichotomy between anti-inflammatory or a pro-inflammatory states of various immune cells [11]. Finally, translational bedside approaches remain challenging to implement due to the lack of available monitoring of the immune response after AIS and the absence of reliable biological and radiological biomarkers that can be easily implemented in daily clinical practice to drive the type and timing of treatments to administer.

This review aims to put into perspective the recent data regarding the neuro- and thrombo-inflammatory response after AIS in a clinical setting and to discuss the added value of newly available clinical imaging and biological "omic" modalities that have refined our current understanding of the spatial organization of the immune system within the CNS physiology and after a brain injury. We will examine recent neuroimaging technologies and their unique capacity to contribute a bona fide spatial "omic" data layer for the characterization of AIS pathogenesis in humans. We will also discuss recent breakthroughs in machine learning

methods for the integration of multimodal clinical and biological omic data to improve the prediction of outcomes after AIS.

Transitioning from an immune-privileged to an active immune organ, through compartments and barriers

The CNS has long been considered an immune-privileged organ, in which any immune response was considered abnormal and systematically associated with injuries and pathological conditions [8]. This concept of immune privilege was first introduced by Medawar, who demonstrated delayed graft rejection after brain parenchymal skin allografts [12]. The immune privilege was also considered for a long time to be the result of poor capacity for antigen presentation and drainage due to the lack of specialized lymphatic vessels [8, 9, 13]. In addition to the lack of lymphatics, the CNS was considered protected from any immune response by the presence of several barriers and anatomical structures, which provide protection by mechanically and chemically restricting the access of immune cells to the CNS (recently reviewed by Buckley and McGavern [8]). Among these barriers and structures, the blood-brain barrier (BBB) and the blood-cerebro-spinal fluid (CSF) barrier restrict the access of cells, cytokines, or other soluble mediators from one immune compartment to another [14]. More specifically, the BBB is a stringent barrier between intracranial blood vessels and the brain, restricting the passage of cells or mediators to the parenchyma due to the complex interplay of highly specialized endothelial cells with astrocytes, pericytes, and microglia [14]. The blood-CSF barrier involves the ChP, which are small, spongy structures that are bathed in the CSF they secrete, located in the ventricular system (mainly the lateral ventricles). The ChP are densely vascular structures with fenestrated capillaries, and they contain a rich and heterogenous population of immune cells [10, 15, 16]. However, exchanges between the ChP and the CSF are tightly regulated by an epithelium with tight junctions, restricting the exchanges (i.e., the blood-CSF barrier) [8]. In addition to these barriers, the meninges and the skull serve as physical layers of protection from the outside [8]. The skull consists of several fused cranial bones, which altogether confers protection to the brain and vascular structures. The meninges consist of three layers (from the outside to the inside: the dura mater, the arachnoid, and the pia mater) [8]. The dura mater (the outermost layer) is made of two sheets, namely the endosteal layer which lines the inner surface of the skull and the meningeal layer which lies against the arachnoid mater. The space between the arachnoid and the pia mater defines the subarachnoid space, which is filled with CSF and contains leptomeningeal arteries and veins. The meninges are highly vascular structures, but although the dura mater contains fenestrated capillaries facilitating the exchanges, the leptomeninges (i.e., arachnoid and pia mater) have tight junctions, restricting access to the CSF and the brain, thereby providing an additional physical barrier [8, 15].

However, our global understanding of the CNS immune system has been revolutionized recently, notably by the demonstration of the presence of meningeal lymphatics vessels (mLVs) by two independent labs [17]. Several works have subsequently described the spatial organization of mLVs along cerebral venous sinuses. They have also provided the topography of immunologic “hot spots” or “hubs” and a detailed characterization of

the mechanisms involved in antigen drainage and antigen presentation by professional antigen-presenting cells from the brain to the cervical lymph nodes [9, 13]. The dogma of immune privilege has also been recently reconsidered by major works that described the close interplay between the immune system and the CNS in physiology, notably through the secretion of several cytokines by specialized immune cells located in the meninges, thereby acting on behavior, memory, or learning [18–20]. Several crucial works have also highlighted the CNS regenerative capacities through immune system action, illustrating that the immune system can have both a deleterious and beneficial impact depending on the timing of immune functions [21–24]. Finally, structures that were once considered solely as barriers were characterized in greater resolution using new technologies, bringing insights regarding their ultrastructure and the immune cells that populate them. As such, the meninges are composed of several immune cells, including innate lymphoid cells, T and B cells, neutrophils, mastocytes, dendritic cells, natural killer cells, monocytes, and macrophages, acting on the brain parenchyma through the secretion of cytokines and chemokines and ready to be quickly mobilized and recruited to the brain parenchyma in case of brain injury [10, 16, 25]. Recent data have also demonstrated a specialized hematopoiesis inside the skull bone marrow, which communicates with the dura mater through direct vascular channels enabling direct neutrophil migration to the parenchyma, bypassing the BBB [26]. Interestingly, this myelopoiesis is the result of a dynamic crosstalk between the drained CSF from the brain's interstitial space acting on the skull bone marrow through these vascular channels [27]. These recent data led to a paradigm shift regarding our understanding of the CNS immune response. As described below, the acute sterile inflammatory response following AIS has long been described as the consequence of a dysfunctional and disrupted BBB, allowing the recruitment of peripheral immune cells to infiltrate the brain parenchyma. However, this immune response must be rediscussed with this immune compartmentalization and barrier organization (ChP, skull bone marrow, and meninges) and the potential beneficial effect of the immune response [24].

Neuro-inflammation and immuno-thrombosis in AIS

A large body of evidence has now shown the contribution of the neuro-inflammatory response in the acute or chronic phases of AIS on the overall outcome and has been reviewed elsewhere [3–5]. Briefly, this robust sterile inflammatory response seems to begin within minutes after the onset of arterial occlusion and is the result of complex and interconnected phenomena, including an excess of oxidative stress production, glutamate excitotoxicity, calcium overload [3, 5, 14], astrocytes, and microglia's response to AIS after engaging pattern recognition receptors, such as the Toll-like receptor (TLR) family, in response to the release of danger-associated molecular patterns by necrotic cells. This results in the activation of the inflammasome pathways (such as the NOD-, LRR-, and pyrin domain-containing protein 3, NLRP3) and pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, IL-17, IL-18, tumor necrosis factor-TNF-α, interferon (IFN)-γ, and chemokine secretion favoring the recruitment of peripheral immune cells to the brain parenchyma through a disrupted BBB [3, 5, 14]. In the following sections, we provide an overview of the temporal and spatial dynamics of the immune response after AIS and discuss the added value of new technologies for the assessment of the immune response.

Temporal dynamics of the immune response after AIS

Several studies have examined the temporal dynamics of immune cell infiltration after AIS using immunohistochemistry at different time points or flow cytometry with conflicting results depending on the AIS model utilized: permanent or transient occlusion of the middle cerebral artery. The acute immune response involves various innate immune cells, including microglia, monocytes, macrophages, dendritic cells, neutrophils [28], and γδ T cells [29–31], as well as adaptive immune cells such as $CD4^+$ and $CD8^+$ T cells with effector functions independent of antigen recognition or TCR co-stimulation in the acute phase [32]. The immune response after AIS is targeted to fight the insult to favor brain repair with the phagocytosis of dead cells and cellular debris, thereby promoting neurogenesis and angiogenesis. However, the sudden activation of the immune system, along with a cytokine storm, the overactivation of the renin angiotensin and sympathetic systems, and an increased oxidative burst, often leads to an excessive and uncontrolled immune response resulting in increased tissue damage. Overall, the acute immune response has been described to worsen the radiological and clinical outcome in AIS, with increased final infarct volume, hemorrhagic transformation, disability, and mortality, in preclinical as well in clinical studies [4, 33, 34]. From a temporal perspective, the acute immune response predominantly involves innate immune cells, as well as adaptive immune cells with effector functions independent of the antigen [3, 32]. On the other hand, the chronic immune response seems to involve primarily the adaptive immune system with effector functions dependent on antigen presentation [3, 35]. In 2009, Gelderblom et al. published a seminal paper in which the temporal dynamics of the immune response were described using immunohistochemistry and flux cytometry in a murine model of AIS [28]. In this work, the accumulation of microglia, macrophages, lymphocytes, and dendritic cells preceded neutrophil recruitment [28]. Interestingly, no lymphocyte activation was observed in the acute phase of the immune response [28]. As previously mentioned, the chronic immune response involves the release of antigen from dead cells which are captured by dendritic cells and presented in peripheral lymphoid organs to specific lymphocytes (T and B cells). These naïve lymphocytes undergo differentiation, clonal expansion, and migration to the brain where the second recognition with the antigen will lead to autoreactive effector B and T cells, their infiltration in the parenchyma, the secretion of several cytokines and perforins, and the secretion of antibodies [3]. This chronic response has been associated with chronic sequelae after stroke and neuropsychiatric symptoms such as depression, anxiety, fatigue, or post-stroke cognitive impairments [3]. In 2015, Doyle et al. demonstrated the infiltration of activated B-lymphocytes weeks after AIS, with isotype switching and IgM, IgA, and IgG antibodies found in the neuropil of adjacent lesions [35]. Interestingly, a strategy aimed at targeting activated B-cells with anti-CD 20 antibodies led to the prevention of cognitive impairments in preclinical models of AIS, underscoring the importance of the adaptive immune system in the chronic phase of AIS [35]. In a clinical setting, Tsai et al. used a single-cell mass cytometry approach to longitudinally profile the peripheral immune response over a 1-year course after AIS [33]. Using new algorithms and machine learning technology (discussed below), they found a robust and prolonged immune response after AIS that occurred in three phases: (1) an acute phase response (day 2) with increased signal transducer and activator of transcription (STAT) 3 signaling in innate immune cells,

(2) an intermediate phase (day 5) with increased cAMP response element-binding (CREB) protein signaling in adaptive cell types, and (3) a late phase (day 90) characterized by the persistence of neutrophils and IgM^+ B cells, in line with previously described preclinical data [33]. Of importance, they also showed an association between the acute inflammatory phase and post-stroke cognitive impairments using the Montreal Cognitive Assessment scale [33]. The work by Tsai et al. was a turning point in translational stroke research, as it showed the feasibility and utility of deep immune profiling using mass cytometry with peripheral venous samples as well as the added value of such technologies for the prediction of important outcomes after AIS [33].

Spatial organization of the acute and chronic immune response after AIS

Interestingly, the recent findings described above regarding the spatial organization of the immune system in the CNS (i.e., meninges, skull bone marrow, and ChP) and the description of functional mLVs led to a reappraisal of the pathophysiological mechanisms involved in the acute and chronic immune response after AIS. An important consideration for the translational approach and the evaluation of future treatments is the possibility that the cellular trafficking from one immune compartment to another may not be uniform for all immune cells [36], although it was long considered that the main access to the brain parenchyma was the disrupted BBB. As an example, in a murine model of AIS, Herisson et al. recently showed that skull bone marrow neutrophils are more likely to migrate to the brain parenchyma when compared to tibial bone marrow neutrophils [26]. Using confocal microscopy, the authors described the migration of neutrophils from the skull bone marrow to the dura mater through direct vascular channels crossing the inner skull and therefore providing access to the brain parenchyma, bypassing the BBB [26]. The chemotaxis of neutrophils was partly explained by a stroma cell-derived factor 1 (SDF-1) gradient, with a significant decline in SDF-1 levels in the skull compared to the brain, favoring the transfer of neutrophils from the skull bone marrow to the dura mater and then to the brain parenchyma [26]. Interestingly, previous works have highlighted the proximity of neutrophils to the meninges. Perez-de-Puig et al. investigated the mechanisms underlying neutrophil infiltration in two AIS models and found a preferential accumulation of neutrophils at the leptomeninges level and brain infiltration of neutrophils from leptomeningeal vessels [37]. In 2012, Gelderblom et al. demonstrated that γδ T cells led to neutrophil infiltration via the IL-17 axis, which induced astrocyte production of CXCL1 [29], known for its neutrophil chemoattractant properties (CXCL1 and receptors CXCR1 or 2) with antagonistic effects on SDF-1 (CXCL12, CXCR4) [38]. They further demonstrated the implication of the well-described IL-23/IL-17 axis for the mobilization of neutrophils, in which conventional type 2 dendritic cells were the main source of IL-23 after AIS, promoting neutrophil infiltration through IL-17 secretion by γδ T cells [30]. Interestingly, the IL-23/IL-17 axis has been extensively studied in other auto-immune or inflammatory disease, such as psoriasis and inflammatory bowel diseases [39]. γδ T cells are crucial innate immune cells, specifically located in peripheral organs, such as the skin, or in serous membranes, such as the peritoneum or the meninges [9, 18, 20]. Put together, these functions are critical as they confer a new and central role to the meninges for the migration of neutrophils in AIS (homing of skull bone marrow neutrophils, mobilization

from the meninges to the brain parenchyma through the IL-23/IL-17 axis with the action of resident dendritic cells and γδ T cells), while neutrophil mobilization was only thought to be mediated through a disrupted BBB.

The spatial organization of the CNS immune response may also be of importance for lymphocyte infiltration. Using in vivo cell tracking of photoactivating T cells, Llovera et al. recently described the ChP as a primary entry site for T cells to the peri-infarct cortex, using a CCL2-CCR2 gradient [40]. Finally, the recent functional characterization of the dural sinuses as a neuro-immune interface, with CSF and interstitial antigen uptake by meningeal antigen-presenting cells and the presentation of CSF antigens to patrolling T cells [13], may explain the chronic immune response described above, although this hypothesis has not been demonstrated in AIS models.

While the parenchymal immune response after AIS involves the recruitment of immune cells through the skull bone marrow, the meninges, the ChP, and blood-born cells recruited through the disrupted BBB, the thrombo-inflammatory response primarily begins within the occluded artery [3, 6] (Fig. 1). Thrombo-inflammation is a complex interplay between the immune system (neutrophils, lymphocytes), endothelial cells, and thrombotic pathways (platelets, activation of the coagulation cascade), altogether resulting in an intravascular thrombosis extension, microcirculatory impairments, and extravasation of neutrophils through the BBB [6]. Thrombo-inflammation begins very early after arterial occlusion, as recently demonstrated by Desilles et al. who observed an accumulation of platelet and leukocytes immediately after middle cerebral artery occlusion most present in the venous compartment, with the formation of microthrombi and parietal fibrin deposits in post-capillary microvessels after 30 min of occlusion [41]. This downstream microvascular thrombosis is an important phenomenon, as it contributes to microcirculatory impairments with futile recanalization. In a further study, the same team demonstrated the formation of venous thrombi despite middle cerebral artery recanalization and leukocyte extravasation through a disrupted BBB [42]. Interestingly, these thrombo-inflammatory mechanisms are associated with microhemorrhages [42] and hemorrhagic transformation within territories without reperfusion [43], a dreaded and often fatal complication following AIS [44]. In the clinical setting, numerous studies have showed the deleterious effect of thrombo-inflammation using different biomarkers as surrogates for neutrophil activation (i.e., myeloperoxidase) [45, 46], or the identification of Neutrophil Extracellular Traps [47, 48]. NETosis is a cell death program initially described in the context of infection, characterized by neutrophil plasma membrane rupture, chromatin decondensation due to histone citrullination, and the extracellular release of DNA forming a web-like structure, favoring aggregates of red blood cells, platelets, and leukocytes and activation of the coagulation cascade [49]. Neutrophil extracellular traps have been identified in the peripheral blood of patients with AIS treated with EVT [48] and in retrieved thrombi, forming an outer shell along with platelets, fibrin, leukocytes, and von Willebrand factor, impairing IVT action [47, 50]. Recently, the Chemical Optimization of Cerebral Embolectomy (CHOICE) trial demonstrated the added value of intra-arterial thrombolysis using alteplase in successfully recanalized patients and showed an 18% increase in the rate of excellent clinical evolution at 3 months [51]. CHOICE highlighted the importance of the

spatial organization of the immune response, by potentially targeting thrombo-inflammation intra-arterially in the target occluded vessel [51].

Altogether, these data emphasize the importance of the temporal and spatial organization in compartments and barriers of the immune response after a CNS insult, such as AIS (Fig. 1). However, several important questions remain unanswered, such as whether the spatial organization of the acute and chronic immune response varies according to the type of AIS (i.e., a lesion involving the cortex versus a deep lesion). In the works discussed above, the immune response is considered deleterious, but several works have also showed beneficial effects of the immune response after a CNS injury, depending on the type of cell involved (T or B regulatory cells [52]), according to the transcriptomic state of the studied cell, or the timing of action [21, 23, 24]. From an evolutionary perspective, teams such as the Schwartz lab or the Kipnis lab have hypothesized a beneficial effect of the immune response after a brain injury, with different mechanisms and pathways involved (i.e., protective auto-immunity, a beneficial effect of monocytes or macrophages with an M2 phenotype) [22, 53], which recently led to a paradigm shift and the assessment of immune checkpoint blockade, including programmed death (PD)-1 or PD-1 ligand (PD-L1) blockade in preclinical models of Alzheimer disease [54] and AIS models [55] showing a beneficial effect of boosting the immune response, paralleling current therapies used in the oncology field. These past few years, most of the treatments evaluated in a clinical setting aimed to reduce the immune response, such as Sphingosine-1-phosphate receptor regulator (FTY720), Natalizumab, IL-1 receptor antagonist, anti-neutrophil (CD11/CD18), or anti ICAM-1 [7]. However, the timing of the immune response seems to be another important factor, as recent data suggested a strategy aimed at dampening the immune response in the acute phase after brain injury had a beneficial effect in the acute phase but a deleterious impact in the chronic phase [23]. Brought into context, these results suggest a strategy aimed at decreasing the immune response in the acute phase and boosting the immune response in the chronic phase could have a clinical benefit. However, it is currently unclear which biological and radiological biomarkers could be assessed in the clinical setting to evaluate this hypothesis. Unfortunately, although several methods may be used to document the temporal dynamics of the immune response at the bedside using CyTOF analysis of peripheral venous or arterial samples [33], the lack of anatomopathological samples and the safety issues of sampling cranial structures such as the meninges preclude any spatial evaluation of the immune response. In the following paragraph, we discuss recent preclinical and clinical developments in neuroimaging that may provide a non-invasive and straightforward evaluation of the immune spatial organization applicable to AIS research.

In vivo assessment of the spatial organization of the CNS immune response to predict outcomes

Several technological advances in neuroimaging have recently allowed an accurate assessment of the morphology of specific immunological structures within the CNS after brain injury, thereby bridging preclinical findings with the clinical setting. These modalities mainly involve the use of positron emission tomography (PET) scan with various radioactive tracers assessing the biochemical and metabolic functions of brain tissues but

also include, more recently, the use of magnetic resonance imaging (MRI), with increasing magnetic fields (1.5T, 3.0T, 7.0T), and the development of new MRI sequences and post-processing software. Interestingly, several studies have tried to assess the associations between radiological findings and the immune response (using either blood samples or PET imaging) and their additional value for outcomes prediction in several brain disorders. In the following section, we provide an overview of neuroimaging techniques in research and clinical practice for assessment of the spatial organization of specialized immune structures within the brain and discuss their value for outcomes prediction in neurological disorders, including AIS. We also provide an overview about computed tomography (Table 1) and MRI (Table 2) techniques and results described above.

Blood-brain barrier

As described above, the BBB represents one of the main gatekeepers of the CNS, limiting access from the peripheral circulation to the brain parenchyma. After AIS, the BBB becomes disrupted, providing an entry point for immune cells to infiltrate the brain parenchyma. In the clinical setting, BBB disruption is strongly associated with early neurological deterioration, intracranial hemorrhage (ICH) occurrence, disability, and mortality [56–59]. However, the specific mechanisms leading to BBB disruption and resulting in edema formation, ICH occurrence, or disability are still largely unknown. BBB disruption has been one of the most studied and well-described immune interfaces in AIS. This is largely explained by its relative ease of evaluation and the diversity of neuroimaging protocols and modalities to explore it. Here, we discuss clinical data to predict the primary outcomes after AIS using BBB disruption evaluation in patients treated with IVT and EVT and provide an overview of the modalities for BBB disruption assessment.

BBB disruption was largely described using injected perfusion imaging [57, 58, 60]. In patients treated solely with IVT, Hom et al. assessed BBB permeability before treatment using perfusion computed tomography imaging. BBB permeability before IVT was highly predictive of symptomatic ICH and malignant edema formation, with 100% sensitivity and 79% specificity [60]. In 2016, Simkins et al. used T2*-weighted MRI perfusion imaging before and after IVT (at 2h and 24h) to show strong associations between focal increased BBB permeability regions and the occurrence of parenchymal hematoma [61]. Interestingly, reperfusion was associated with decreased BBB permeability, whereas sustained ischemia or the absence of reperfusion was associated with increased BBB permeability and PH occurrence [61]. These findings were important as they not only illustrated the dynamic nature of BBB permeability and its potential for reversibility, but also its prognostic value in terms of outcomes prediction [61].

In the endovascular era, increased BBB permeability has also been described as a strong predictor of worse outcomes. Leigh et al. showed that BBB disruption characterized by an increased BBB permeability assessed on a T2*-weighted perfusion imaging was strongly associated with ICH occurrence and ICH severity [58]. Non-contrast perfusion imaging was also used by Yu et al., using arterial spin labeling (ASL) to evaluate perfusion after AIS at various time points [62]. Maps generated showed associations between hyperperfusion on ASL and ICH after treatment [62]. More recently, Ng et al. used T2*-weighted

contrast-enhanced perfusion imaging 24 h after EVT and analyzed the associations between microvascular dysfunction in BBB disruption with ICH occurrence and edema formation in 238 patients [63]. Interestingly, they found that BBB permeability was associated with worse outcomes and increased cerebral edema formation, but that persistent tissue hypoperfusion (and not hyperperfusion) 24 h after reperfusion was also associated with worse cerebral edema, even in successfully reperfused patients [63]. These data are important, as they question the pathophysiological mechanisms underlying BBB disruption.

Another modality to assess BBB disruption is the use of cone-beam computed tomography following EVT in the angiographic suite, using the iodinated contrast injected during the endovascular procedure for the assessment of contrast staining (i.e., BBB disruption) [56, 64]. With such techniques, Desilles et al. and Rouchaux et al. found that BBB disruption assessed after EVT was strongly associated with disability, ICH occurrence, and mortality [56, 64]. This technique is particularly interesting from a research and therapeutic perspective, as it is widely available, easy to interpret, and could also be used to tailor the therapeutic management immediately after reperfusion.

In 2004, Warach and Latour introduced the concept of the hyperintense acute reperfusion marker (HARM), detected on a post-contrast 3D Fluid Attenuated inversion recovery (FLAIR) sequence as enhancement of the CSF space [65]. Being a possible consequence of BBB disruption rather than a direct visualization of the disrupted BBB, HARM seems to be a good predictor of worse outcomes [65]. The same team also evaluated the relationship between baseline neutrophil, lymphocyte, and monocyte counts with BBB disruption, using HARM on a 3D T2 FLAIR sequence after AIS [66]. The absolute number of monocytes was significantly higher in patients presenting with HARM, underscoring the link between BBB disruption and neuroinflammation after stroke [66].

These studies highlight the prognostic value of BBB disruption or increased BBB permeability after AIS to predict major outcomes and underscore the importance of evaluating BBB pathophysiology after AIS. Although one study described an association between monocyte count and BBB disruption [66], further studies are needed to evaluate multiple inflammatory markers with BBB disruption, using single-cell technologies such as CyTOF. These studies could unveil undescribed mechanisms leading to and explaining the consequences of BBB disruption.

Choroid plexus

As discussed above, the ChP play a central role in the trafficking of immune cells in the CNS.

Different MRI sequences, techniques, and contrast agents have been evaluated for ChP assessment in CNS disorders: 3D pre-contrast and post-contrast T1-weighted sequences have been shown to provide an accurate assessment of ChP volume and contrast enhancement, while 3D pre/post-contrast FLAIR imaging may provide a more sensitive assessment of subtle gadolinium enhancement compared to 3D T1-weighted imaging [67]. ChP permeability has been mostly assessed using dynamic susceptibility-contrast (T2*-weighted) or dynamic contrast enhancement (T1-weighted) MRI perfusion imaging [67].

Several parameters can be obtained after post-processing, such as water efflux rate constant (K_{co}) or the K_{trans} (measure of contrast agent extravasation to the interstitial space) [67, 68]. Non-contrast and non-invasive perfusion imaging using ASL, in which water is used as a contrast agent, have also been used to evaluate ChP permeability [69]. Interestingly, a substantial number of studies have described associations between an alteration of ChP morphology (typically enlargement) or a functional anomaly (contrast enhancement, increased or decreased permeability) with neuro-degenerative disorders, such as Alzheimer disease; inflammatory conditions, such as multiple sclerosis (MS) or neuromyelitis optica spectrum disorders (NMOSD); hydrocephalus; stroke; neuropsychiatric disorders, such as schizophrenia and depression; and aging [70–75]. Using pre- and post-gadolinium 3D-T1 and FLAIR-weighted imaging, Klistorner et al. found that baseline ChP volume was larger in patients suffering from MS compared to healthy patients, and that baseline ChP volume was predictive of subsequent chronic lesion expansion [70]. In addition, Fleischer et al. illustrated the prognostic value of ChP assessment in neuro-inflammatory conditions by showing a strong association between ChP enlargement with MS activity and a high efficacy of treatment (natalizumab) to prevent ChP enlargement [76]. This result was crucial, as it underscored the value of monitoring neuroimmune structures with MRI for the evaluation or the prediction of treatment response in neuroinflammatory conditions [76]. Ricigliano et al. also showed ChP enlargement in patients with MS along with a strong correlation with lesion load [72]. Interestingly, ChP volume predicted annualized relapse rate in patients with MS [72]. Further evidence suggesting a link between ChP morphological alteration and neuroinflammation was provided by Huang et al., in which enlarged ChP were associated with higher levels of salivary kynurenic acid [74]. Interestingly, the kynurenine pathway is implicated in the neuroinflammatory response and has recently emerged as a reliable biomarker for post-stroke cognitive impairments in a preclinical model of AIS and in the clinical setting [77]. Unfortunately, only one clinical study assessed alterations of ChP after AIS [71]. In this study, ChP volume was assessed in the lateral ventricles of patients with AIS and healthy controls at three time points (baseline, 3 months, and 12 months), using an automatic segmentation tool on 3D T1-weighted and 3D FLAIR sequences [71]. Larger ChP were found in patients with AIS compared to healthy patients, at all time points, but ChP did not change over the first year after AIS and there was no correlation with infarct volume or clinical severity [71]. Unfortunately, no analysis was performed regarding the association between ChP volume after AIS and long-term outcomes. Thus, these preliminary data are important, but further studies will be needed to evaluate the prognostic value of ChP MRI assessment after AIS to predict outcomes.

Meningeal lymphatics

Since the description and functional characterization of mLVs in 2015 [17], several teams have tried to characterize these mLVs in vivo in humans, using several types of neuro-imaging modalities. Absinta et al. were the first to describe the mLV in vivo [78]. In this study, the authors compared two contrast agents: gadobutrol (which has a high propensity to extravasate across a permeable endothelial barrier) versus gadofosveset (which is restricted to the vasculature) [78]. Using different types of sequences in T1-weighted and FLAIR, they described the topography of the mLVs which recapitulated the mLVs system described in rodents (i.e., mLVs running alongside dural venous sinuses) [78]. Using an intrathecal

administration of gadobutrol, in a landmark study, Ringstad and Eide evaluated the clearance pathways of human dural lymphatics in vivo using MRI [79]. In this study, 3D T1 and T2 FLAIR weighted sequences prior to contrast injection were used, with a longitudinal assessment performed at 3, 6, 24, and 48 h [79]. This study provided for the first time the dynamics of CSF direct efflux to parasagittal dura mater in humans along the superior sagittal sinus [79]. Recently, Jacob et al. achieved an outstanding work describing and comparing the anatomical and functional organization of mLVs drainage circuits in mice and humans [80]. The MRI protocol for this study included an intravenous injection of gadobutrol prior to MRI. The venous anatomy was mapped with a magnetic resonance venography sequence, allowing visualization of venous sinuses, veins, and venules [80]. This was followed by a 3D T1-weighted sequence 15 min after contrast agent injection [80]. This latter sequence allowed a different approach for mLVs assessment, with the stratification of the vessels according to their flow: slow flow circuits for mLVs and faster flows for arteries, veins, and venules [80]. Jacob et al. generated 3D maps of outstanding quality and spatial resolution, exhibiting the presence of mLVs in vivo, along the superior sagittal, straight, transverse, and sigmoid sinus, but also in the cavernous sinus, exiting the intracranial space through neural foramina to connect with cervical lymph nodes [80]. Since then, several papers aimed to provide an in vivo assessment using MRI of mLVS in different neurological disorders and found associations between mLVs impairments and MS, Parkinson disease, Alzheimer disease, glioblastoma, and aging [81–83]. Using dynamic contrast enhancement MRI, Wang et al. found slower flow through mLVs along the superior sagittal sinus in patients with NMOSD presenting with an acute attack, compared to patients with NMOSD in the chronic phase [83]. This reduced flow in mLVs predicted clinical severity assessed by the expanded disability status scale [83]. With the same MRI protocol, Ding et al. assessed mLVs flow in idiopathic Parkinson disease [82]. Patients with Parkinson disease exhibited reduced flow through mLVs along the main venous sinuses and a delay cervical lymph node perfusion [82]. Only one study assessed the functional relevance of mLVs in AIS [84]. In this study, Yanev et al. found a different impact of the mLV according to the AIS model [84]. Lymphangiogenesis was induced in a photothrombotic AIS model but not after a transient middle cerebral artery occlusion model [84]. Interestingly, using knock-out mice for vascular endothelial growth factor receptor 3 (VEGFR 3), a receptor involved in lymphangiogenesis, the authors found a strong association between the lack of lymphangiogenesis and stroke outcomes [84]. This study highlights the different pathophysiology of the immune response according to the stroke model and underscores the need of in vivo data in humans. Unfortunately, no study has ever evaluated the mLVs after AIS in humans yet, but studies are ongoing using these MRI protocols.

Brain parenchyma

Brain parenchymal inflammation after AIS has largely been assessed using PET imaging [4]. Nicely reviewed by Shi et al., PET using a radioactive ligand of the translocator protein highly expressed by activated microglia ([11C] PK11195-PET) has repeatedly revealed microglia activation at the lesion’s border and more distally [4, 85]. Also using [11C]PK11195 PET imaging in patients presenting with chronic middle cerebral artery AIS (2 to 24 months), Pappata et al. showed increased microglial activation in the thalamus ipsilateral to the AIS [86]. Price et al. assessed longitudinal microglial activation using

[11C]PK11195-PET and found minimal microglial activation during the first 72 h after middle cerebral artery AIS compared to healthy individuals, but microglial activation was reported in the infarct core, peri-infarct, and contralateral hemisphere for up to 30 days [87]. In a prospective study, Morris et al. evaluated the relationship between neuronal loss and microglial activation after AIS, using a multimodal radiological approach with brain MRI and perfusion imaging to delineate the ischemic penumbra and the final infarct volume and PET with [11C] flumazenil and [11C] PK11195 to assess neuronal loss and microglial activation, respectively [88]. This study provided evidence, for the first time, of both neuronal loss and microglial activation in the ipsilateral non-infarcted zone and neuronal loss without microglial activation in the surviving penumbra after reperfusion [88]. However, although PET imaging may bring important pathophysiological insights, its limitations include increased tracer metabolites passage through the disruptive BBB, resulting in increased signal and its generalization in everyday practice is challenging, as illustrated by the small sample size in these studies.

Recently, Gauberti et al. coined the term “inflammatory penumbra,” in which intact brain regions are the site of a pronounced inflammatory response, with T-cells diapedesis triggering secondary neuronal loss [89]. Gauberti et al. recently reviewed the preclinical and clinical evidence of the inflammatory response after AIS and innovative neuroimaging methods including MRI of adhesion molecules, such as VCAM-1 [89]. They also discussed the development of new contrast agents, microsized particles of iron oxide, which possess a superparamagnetic propriety evaluable on T2* and T1-weighted MRI sequences (hypointense signal in T2* and hyperintense signal in T1 sequence) and the conjugation of microsized particles of iron oxide with specific adhesion molecules (VCAM-1) [89]. These techniques are attractive, as they could be more generalizable and accessible in clinical practice to noninvasively monitor and guide the selection of patients for the evaluation of future treatments.

Skull bone marrow

Since the identification of direct vascular channels connecting the skull bone marrow to the dura mater, an important amount of preclinical data has emerged to describe the ontogeny of resident immune cells, their spatial organization, and their involvement in different neurological disorders [26, 27]. In humans, Ringstad and Eide showed a transdural efflux of CSF to the skull bone marrow [90]. In this seminal work, the authors described tracer enrichment within the diploe of the skull bone marrow near the parasagittal dura [90]. This work was critical, as it was the first in vivo demonstration and characterization of the communications between the dural lymphatic meningeal system and the skull bone marrow [90], underscoring the possible impact of the skull bone marrow on the brain’s immune surveillance. Jacob et al. also described perforating venules and slow-flow channels (i.e., lymphatic vessels) crossing the skull, thereby connecting to superficial intracalvaria and subcutaneous lakes [80]. Unfortunately, data are lacking regarding the involvement of the skull bone marrow in a clinical setting of AIS. Thus, further studies will be needed to characterize its prognostic significance and its relationship with BBB disruption, mLVs, or ChP enlargement.

Integrating clinical imaging into “multi-omic” models to predict AIS clinical outcomes

Advances in high content “omic” technologies have transformed our ability to study complex pathophysiological processes, such as stroke, with unprecedented depth and cellular resolution. In particular, the analysis of circulating immune cells using either scRNAseq or single-cell proteomic platforms, such as mass cytometry, is a powerful approach to identify the fingerprints of local inflammatory processes in a readily accessible immune compartment. As previously mentioned, a single-cell mass cytometry study of patients recovering after an AIS identified acute and long-term immune adaptations (including elevated CREB and P38 signaling in T cell subsets and increased STAT3 signaling in innate immune cells) that were predictive of functional outcome and long-term-sequelae such as cognitive decline [33]. The results are partially corroborated by a recent murine scRNA-seq study, showing enrichment of immune-related genes after AIS, including genes related to T cell activation, chemotaxis and leukocyte migration, cytokine secretion, and inflammatory signaling pathways such as ERK/MAPK, type I interferon, and cAMP/cGMP-mediated signaling [91].

In parallel with peripheral immune cell profiling, single-cell technologies such as imaging mass cytometry and spatial transcriptomics enable a highly multiplex spatial exploration of local pathophysiological mechanisms in situ. This allows for unparalleled insight into local microenvironments that may determine and contribute to pathological disease progression, as demonstrated in the context of cancer [92], infections [93], and autoimmunity [94]. As such, the implementation of high-content spatial “omic” technologies to study local immune responses in patients with AIS in future studies is a highly promising approach to identify features indicative of local immunological dysfunction that are associated with, and perhaps driving, the pathogenesis of AIS.

The bioinformatics analysis necessary to integrate multiple “omics” datasets is complex, as such datasets are high-dimensional and can vary in size and signal-to-noise content. Traditional univariate or multivariate approaches are ill-adapted for biomarker identification and predictive modeling on omic datasets due to the surfeit of features that characteristically exceeds the number of samples [95, 96]. To counteract overfitting of the modeling approach and to ensure a higher generalizability of the selected features, algorithms such as least absolute shrinkage and selection operator (LASSO) or the Elastic-Net introduce sparsity through regularization for the analysis of high-dimensional data [96, 97]. These models use an additional constraint on the structure of the regression coefficient that coerces many model coefficients to zero, thereby producing a final model fit that yields smaller and more interpretable subsets of covariates. Such regularized regression algorithms have also been adapted to enable the incorporation of prior biological knowledge, thereby improving predictive performance [98–100]. However, the feature selection often remains highly variable over many iterations of model fitting and requires further post-hoc analyses to gain interpretability. Random forest modeling represents another prediction approach for omics data, but its lack of embedded feature selection inherently hinders biological interpretability.

For integrative multi-omic analyses, predictive models can be built either on each data layer individually (late fusion) or on one concatenated set of all omics data (early fusion) [98, 99]. The latter allows for inter-omic crosstalk, while a late fusion approach improves the balance between data layers. In addition, recent advances in cooperative multiview learning leverage the underlying relationship between omics dataset by inducing an “agreement” penalty, thereby enabling a continuous optimization between early and late fusion approaches and improving predictive performance [100].

By analogy to other omic modalities, multimodal clinical imaging also provides high-content spatial information that can be integrated into multi-omic models, as demonstrated in studies for various cancer types [101–103]. After identification and labeling of regions of interest on the computed tomography, MRI, or PET images, radiomics features such as geometric shape, signal intensity, and texture features can be extracted and used as an omic data layer. Radiomics can offer unparalleled anatomical and functional characterization of AIS pathogenesis and complement single-cell omic data by echoing mechanisms that drive the pathogenesis of BBB leakage, TI, and hemorrhagic transformation after AIS. Integrative analysis of clinical imaging and single-cell omics will allow interrogating multiple interconnected biological systems and identification of otherwise unrecognized pathophysiological crosstalk (Fig. 2). This type of analysis will allow researchers to identify links between imaging and molecular features at a single cell resolution in relation to outcomes of interest. From a diagnostic perspective, an integrated analysis can also reveal unique biomarkers from several biological domains that provide higher predictive power when combined. With the accelerating development of new machine learning algorithms adapted to the analysis of complex clinical and biological data, integration of clinical imaging and single-cell omic data is an ambitious yet reachable step for the discovery of novel, accurate, and actionable biomarkers of clinical outcomes in patients with AIS (Fig. 2).

Conclusion and perspective

The neuro-inflammatory and thrombo-inflammatory responses to AIS have been exhaustively studied in preclinical models of AIS, leading to the design of clinical trials of several promising immunomodulatory drugs that largely failed to show a meaningful clinical effect. Challenges in translating preclinical findings to the clinical setting may be partly explained by the underappreciation of patient-to-patient variability in drug response, driven in part by cell-type or immune compartment-specific differences in patients’ inflammatory responses to AIS. Notably, the spatial re-organization of various immune compartments after an AIS is yet to be comprehensively characterized, and clinical studies investigating the prognostic significance of these structures are critically lacking.

We provided an overview of the thrombo- and neuro-inflammation events occurring in the skull bone marrow, the mLVs, the ChP, and brain parenchyma after an AIS and discussed recent advances in clinically available neuroimaging and single-cell omic modalities that enable observation of the spatial organization of these cellular events in patients. We also discussed emerging machine learning methods that allow integration of spatial, radiological, single-cell, and clinical data into holistic multi-omic frameworks to capture clinically relevant biological variability differentiating patients suffering from an AIS. Implementing

these multi-omic approaches to identify robust and biologically plausible biomarkers forms a new frontier in the development of targeted immune-modulatory interventions that can be tailored to patient-specific inflammatory states predictive of AIS clinical outcomes.

Acknowledgements

We thank Dyani Gaudilliere for providing her scientific input and editing the manuscript.

Funding

This work was supported in part by the National Institute of Health (NIH) R35GM137936, P01HD106414 (BG), the Precision Health and Integrated Diagnosis Center, and the Center for Human Systems Immunology at Stanford (BG). This work was also supported by INSERM and by public grants overseen by the French National Research Agency (ANR) as part of the Investments for the Future program (PIA) under grant agreement No. ANR-18-RHUS-0001 (RHU Booster) and ANR-ETHERISCH.

Conflict of interest

MM declares institutional fees for teaching presentations from Boerhinger Ingelheim, Medtronic, Amgen, and consulting fees from Boerhinger Ingelheim, Acticor Biotech, and Air liquide. MM received a grant from the French National Research Agency (ANR) as part of the Investments for the future program (PIA) (grant agreement no. ANR-18-RHUS-0001) for the BOOSTER (Brain clOt persOnalized therapeutic Strategies for sTroke Emergent Reperfusion) consortium. BG declares consulting fees from Surge2surgery. JMO declares institutional fees for teaching presentation for Boehringer Ingelheim and Bristol Myers Squibb; Consulting fees from Abbvie, Acticor, and Bioxodes.

Abbreviations

AIS	Acute ischemic stroke
ASL	Arterial spin labeling
BBB	Blood-brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
ChP	Choroid plexus
EVT	Endovascular therapy
FLAIR	Fluid attenuated inversion recovery
HARM	Hyperintense acute reperfusion marker
ICH	Intracranial hemorrhage
IL	Interleukin
IVT	Intravenous thrombolysis
mLVs	Meningeal lymphatic vessels
MS	Multiple sclerosis
MRI	Magnetic resonance imaging

NMOSD	Neuromyelitis optica spectrum disorders
PET	Positron emission tomography
SDF-1	Stroma cell-derived factor 1

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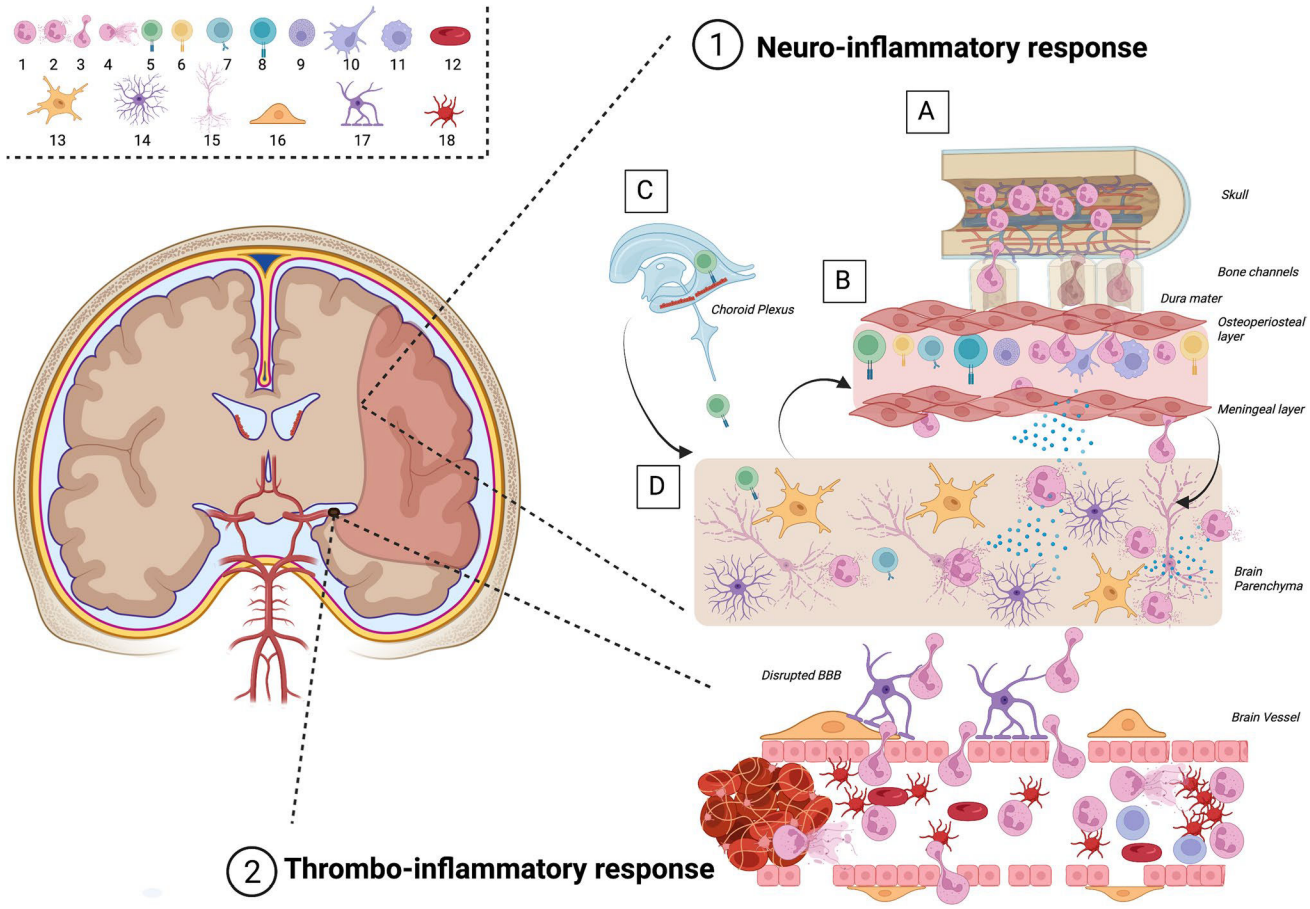

Fig. 1.

Spatial organization of the neuro-inflammatory and thrombo-inflammatory response after an acute ischemic stroke. (1) The neuro-inflammatory response after an acute ischemic stroke. (A) The acute ischemic stroke triggers a myelopoiesis within the skull bone marrow, allowing the recruitment and infiltration of neutrophils from the skull bone marrow to the meninges, through vascular channels. This recruitment is driven by an SDF-1 gradient between the skull bone marrow and the brain parenchyma. (B) The dura mater surrounding the brain parenchyma, homing a wide variety of immune cells. In the setting of AIS, neutrophils accumulate in the dura mater and the leptomeninges, to infiltrate brain parenchyma. In addition, the dura mater hosts dendritic cells, which may stimulate gamma delta lymphocytes through the IL-23–IL-17 axis. Gamma delta-IL-17 secreting cells are key players in the chemotaxis of neutrophils to the ischemic parenchyma. (C) The plexus choroid inside the ventricular system (here, the lateral ventricle). The plexus choroid are immune structures involved in the trafficking of several immune cells in health and in disease (monocytes, lymphocytes, etc.) and the site of the blood-CSF barrier. After stroke, several studies described a lymphocyte migration pathway through the plexus choroid, allowing recruitment and infiltration to the ischemic parenchyma. (D) The ischemic brain parenchyma. Resident immune cells, such as microglia, are being reactive to after ischemic stroke and secrete several cytokines and chemokines that promote skull bone marrow, meninges, and choroid plexus immune cell infiltration. Overall, the neuro-inflammatory

response may worsen parenchymal lesions, leading to neuronal death, hemorrhagic transformation, and disability. That said, the neuro-inflammatory response has also the aim to clear dead cells and cellular debris through phagocytosis, as a preliminary step for brain repair (not depicted). (2) The thrombo-inflammatory response. From a spatial perspective, the thrombo-inflammatory response begins intravascularly, right after the onset of arterial occlusion. The blood clot is the site of aggregated blood cells, fibrinogen, activated platelets, and neutrophils. The blood-brain barrier, a physiological structure comprising specialized endothelial cells, pericytes, and astrocytes end feet, is disrupted, allowing the infiltration of activated neutrophils to the brain parenchyma. Activating neutrophils release several granules containing proteins, such as MMP-9, elastase, and myeloperoxidase, that may increase parenchymal damage and favor hemorrhagic transformation. In addition, downstream microvascular thrombosis is the result of increased NETosis, platelets and neutrophils, platelets, and lymphocyte aggregates, leading to microvascular impairments. (1) Neutrophil; (2) degranulating neutrophils; (3) migrating neutrophil; (4) NETosis; (5) CD4+ lymphocyte; (6) Gamma-Delta lymphocyte; (7) B lymphocyte, (8) CD8+ lymphocyte; (9) mast cell; (10) dendritic cell; (11) macrophage; (12) red blood cell; (13) microglia reactive to AIS, (14) activated astrocyte; (15) dying neuron; (16) pericyte; (17) astrocyte end feet participating in the blood-brain barrier; (18) activated platelet

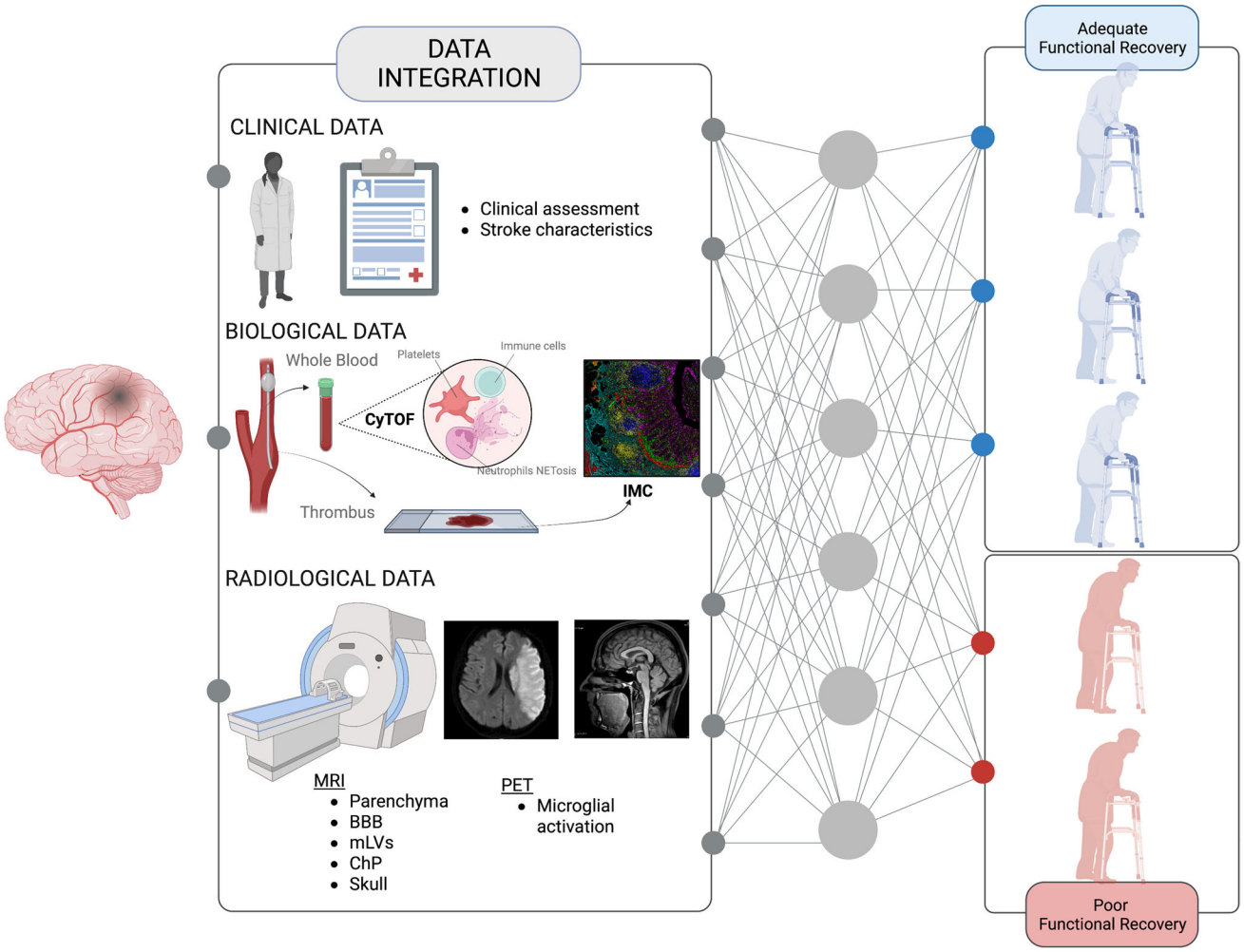

Fig. 2.

Integration of multiple omic and clinical imaging data for the prediction of patient outcomes after acute ischemic stroke. High-dimensional clinical and biological data is collected in patients suffering from IS across multiple omic, clinical, and clinical imaging data modalities. Omic data modalities can include single-cell transcriptomic (scRNAseq), proteomic (CyTOF, mass cytometry), bulk proteomic or metabolomic data, as well as high-dimensional transcriptomic or proteomic imaging (e.g., imaging mass cytometry) data. Advanced machine learning methods such as stacked generalization or multiview cooperative learning allow integration of multi-omic and clinical data to identify robust predictive models of IS clinical outcomes that combine multiple biomarkers from individual data layers. Clinical data can be collected in the form of patient demographics (age, gender, smoking history, CVD, etc.) or stroke characteristics (size and location). Biological data can be obtained by studying the peripheral immune system and any crosstalk with the central immune system via suspension mass cytometry or high-resolution analysis of the occlusive thrombus or brain parenchyma using imaging mass cytometry. Radiologic data can be obtained using MRI or PET. These multi-omic data can be integrated by advanced machine learning methods to predict functional outcome. Figures created with biorender.com

Table 1

Brain computed tomography as a new spatial “omic” in ischemic stroke: technique and results

Computed tomography (i.e., scan)		
Brain computed tomography (CT) is the most frequently used and widespread neuro-imaging modality. This imaging is based on the measurement of X-ray absorption by the tissues and leads to a cross-sectional imaging of the brain. The brain CT can be performed without intravenous injection for the assessment of the skull (for example, fracture) and the brain parenchyma (diagnostic of an AIS, an ICH) and after intravenous injection of contrast. Main results are described according to the observed or measured density of the anatomical structure (hypodense versus hyperdense) and the enhancement after contrast injection		
Name of the neuro-imaging modality	***Technique (rational)***	***Main results and anatomical structures assessed***
Non-contrast brain CT	Fast, available 24h/24h, evaluation of the brain parenchyma in the 3 dimensions of space (axial, sagittal, and coronal)	- AIS, as a hypodensity within a vascular territory - ICH, as a spontaneous (i.e., without injection) hyperdensity
Cone-beam CT	Cone-beam CT is a type of brain CT performed in the angiographic suite after EVT. Providing less accurate images than the conventional brain CT, the cone-beam CT in the angiographic suite provides a cross-sectional evaluation of the brain before and after contrast injection	- ICH, as a spontaneous hyperdensity - Assessment of the BBB: Contrast injection is used during EVT; therefore, the cone-beam CT performed after EVT is considered injected. Contrast enhancement in the brain parenchyma seen on the cone-beam after EVT is considered as BBB rupture, as this contrast should stay intravascularly and not leak in the brain parenchyma, in case of a healthy BBB
Brain CT after contrast injection	CT angiography (CTA) is performed after contrast injection, with an early acquisition of the CT at the arterial time (initiation of the protocol when the contrast is seen in the aorta)	CTA allows an assessment of the brain arteries and the diagnosis of arterial occlusions
	Perfusion CT is also performed after contrast injection, but with a longer acquisition time for the assessment of the microcirculation. Several quantitative parameters can be extracted from this sequence, to evaluate the microcirculation at the capillary level and quantify either hypoperfusion or hyperperfusion according to the delay or the too prompt arrival of the contrast agent through the microcirculation	- AIS, as a hypoperfusion area distal to an arterial thrombus. The perfusion allows the characterization of the ischemic core and the ischemic penumbra, according to the quantification of the cerebral blood flow and the mean transit time - Assessment of the microcirculation: Perfusion can be performed after successful recanalization by EVT to characterize either hyperperfusion or hypoperfusion within the ischemic core. Hypoperfusion within the ischemic core despite successful recanalization led to the definition of the “no-reflow phenomenon” (i.e., recanalization, without reperfusion). Hyperperfusion after recanalization has been described to be associated with hemorrhagic transformation

Table 2

Brain magnetic resonance imaging as a new spatial "omic" in ischemic stroke: technique and results

Magnetic resonance imaging (MRI)		
Brain magnetic resonance imaging (MRI) is the most advanced neuro-imaging modality available at the bedside. Using high magnetic field gradients (1.5, 3, or 7T), several different types of acquisition can be performed in a same patient to assess the brain parenchyma, the vasculature, and the brain's border (meninges). According to the setting of the acquisition, different images are performed in which the signal of water, fat, or blood can be reversed, deleted, or saturated. Brain MRI can be performed either without contrast injection or after intravenous gadolinium injection (i.e., contrast agent). These acquisitions include a 2D (axial) or 3D (axial, sagittal, and coronal) assessment of the brain		
Name of the neuro-imaging modality	***Technique (rational)***	***Main results and anatomical structures assessed***
MRI without intravenous contrast injection	T1 weighted image (2D or 3D)	2D-T1 is useful for brain parenchyma analysis. Based on the signal obtained in T1, a lesion or a structure may be described as hypointense (CSF, water, air) or hyperintense (fat, blood) 3D-T1 is the main anatomical acquisition, allowing a 3D assessment of the brain with a good delineation of the gray and white matter. In a T1-weighted acquisition, the gray matter is darker than the gray matter
	T2*	T2* (or T2 star or gradient echo) is an important MRI acquisition highly sensitive for hemorrhage detection or hemosiderin deposition (hypointense signal). The basis of the acquisition is also used for injected perfusion imaging, as explained below
	Fluid-attenuated inversion recovery (FLAIR, 2D, or 3D)	This acquisition aims at suppressing CSF signal to increase the detection of intraparenchymal and periventricular lesions. FLAIR can be used in axial (2D) and in 3D. FLAIR is extremely used in AIS neuro-imaging protocols, as it allows an estimate of the final stroke volume (hyperintense signal), an estimate of AIS onset in case of unknown onset
	Arterial spin labeling (ASL)	ASL is a perfusion imaging without any intravenous contrast agent injection and uses the labeling of the blood water content (i.e., the protons of water are used as contrast agent) AIS: as a perfusion acquisition, the ASL allows the visualization of the ischemic penumbra as a hypoperfusion area potentially responsible for the symptoms (hyposignal) After recanalization by EVT, ASL can be used to look for either hyperperfusion areas at risk of ICH, or hypoperfusion ("no-reflow phenomenon") Permeability: ASL can also be used to assess the permeability of different structures, such as the BBB (i.e., disrupted BBB after AIS) or the ChP
MRI with intravenous contrast injection	T1-weighted image (3D)	3D T1 weighted acquisitions after gadolinium injection are used to look for contrast enhancement. In the setting of AIS, injected 3D T1 weighted acquisition may be used to evaluate the volume and permeability of the ChP, and also the permeability of the BBB. Indeed, in case of the sealed and healthy BBB, gadolinium (contrast agent) does not cross the BBB. Therefore, intraparenchymal gadolinium enhancement after AIS seen on 3D-T1 acquisitions is an indirect sign of BBB rupture Injected 3D T1-weighted are also useful for the assessment of meningeal enhancement (i.e., inflammation, tumor infiltration), and more recently the assessment of mLVs
	FLAIR (3D)	3D FLAIR acquisitions after gadolinium injection may also be useful for the detection of intraparenchymal and meningeal enhancement, and the evaluation of ChP volume. This acquisition was also recently used to delineate the anatomy of the mLVs in the wall of dural sinuses
	T1 weighted perfusion	T1-weighted perfusion (also known as dynamic contrast enhanced or DCE perfusion) is a type of MRI injected perfusion acquisition that allows an evaluation of the permeability (i.e., extravasation) by measuring different constant after post-processing (such as Ktrans). Recently, this acquisition was described for the assessment of the ChP permeability

Magnetic resonance imaging (MRI)		
	T2* weighted perfusion	T2*-weighted perfusion (also known as dynamic susceptibility weighted contrast or DSC perfusion) is the most used perfusion acquisition in the setting of AIS, to delineate the ischemic core and the ischemic penumbra (hypoperfusion) by measuring specific parameters (cerebral blood volume, cerebral blood flow, mean transit time) after post-processing T2* perfusion also allows the assessment of the microcirculation and the BBB after recanalization (hypoperfusion for the “no-reflow,” hyperperfusion at risk of ICH)