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New Concept in Stroke Diagnosis

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Abstract: Stroke is a life-threatening event that is expected to more than double over the next 40 years. Approximately 85% of strokes are ischemic in nature and result from thromboembolic occlusion of a major cerebral artery or its branches. One of the diagnostic methods for detection of the cerebral ischemia is the gadolinium-enhanced MRI imaging. It is mainly used in patients to detect brain tissue damaged by an ischemic stroke and brain hemorrhage. These techniques are expensive, require sophisticated machines and are time consuming. A recent study in acute stroke patients showed gadolinium leakage into ocular structures (GLOS) during MRI imaging with gadolinium administration. The results indicate that at 2 hours after administration of the contrast agent, GLOS was more common in the aqueous chamber alone, compared to the vitreous chamber with increasing amount in 24 hours after the administration of the contrast agent. This could be due to disruption of blood-ocular barrier similar to the disruption of blood-brain barrier in acute stroke. A new approach to diagnosis of acute stroke and transient ischemic attack (TIA) is through the detection of sodium fluorescein contrast agent in the eye by i.v. injection. The agent is safe and is used routinely in eye fluorescein angiography. Fluorescein fluorescence occurs at the visible wavelengths and can be detected by fluorescein angiography camera. The fluorescein angiography camera prices are affordable for any medical clinic. The innovation of this method is to leverage the eye as the window to the brain. The method can potentially detect acute stroke and TIA without MRI. This can have a far-reaching impact on the healthcare system. The eventual feature of the device will be portability and simplicity of operation that can be used by a medical technician in medical office, emergency outfits and even in ambulances given the portability.

Keywords: Acute Ischemia; stroke; diagnosis; eye; fluorescein angiography; MRI Imaging

1 Introduction

Stroke is a medical emergency that occurs when blood supply to the brain is interrupted which results in death of neurons that can cause permanent damage to the brain and possibly death. Stroke affects 7M Americans with ~800,000 new cases occurring each year [1]. Stroke is also the leading cause of long-term disability which leads to diminished patient quality of life (QOL) with large accompanying healthcare costs (~$34 B/yr.) [1]. The prevalence of stroke in the US is seen most prominently in women, minorities, such as African Americans, and the increasing elderly population [1,2]. Multiple factors contribute to stroke incidence including: Cardiac rhythm disorders (e.g., atrial fibrillation ~2-6M U.S. currently) [3,4], diabetes mellitus (18-23M) [5], high cholesterol and/or blood pressure (76-103M) [6], tobacco usage (70M) [7], end-stage renal disease (0.5M) [8], and physical inactivity (102M) [9]. Stroke prevention is difficult to achieve because certain factors, such as tobacco usage and physical activity are out of clinical control, while other factors, like AF, are treatable with therapies such as oral anticoagulants. These treatments only reduce

the risk, however, but do not eliminate stroke [1]. In addition, recent transcatheter procedures such as transcatheter aortic valve implants have increased the risk of stroke [10].

Approximately 85% of strokes are ischemic in nature and result from thromboembolic occlusion of a major cerebral artery or its branches [11]. Atherosclerosis is one of the most common causes of ischemic stroke worldwide [12,13] and is associated with a high rate of recurrence [14]. Transient ischemic attacks (TIAs) cause similar symptoms, but the blockage of blood flow to the brain is temporary. About one-third of people who have a TIA will have a stroke within one year [15]. Chronic ischemia may result in a form of dementia called vascular dementia. It appears to result from damage to the white matter which may be preceded by disruption of the blood-brain barrier [16,17]. Sub-clinical or silent stroke is a stroke that does not have any outward symptoms associated with the stroke and the patient is typically unaware they have suffered a stroke. In a broad study in 1998, about 11 million people were estimated to have experienced silent stroke [18]. The silent strokes are only discovered when using brain imaging.

In this paper, we discuss the established practices of the acute stroke diagnosis and potential future direction. We also propose how the TIA could be differentiated from the acute stroke without MRI imaging. We will not address the chronic ischemia or the silent stroke since the patient is asymptomatic.

2 Diagnostic Methods

2.1 Current Practice

The most commonly used imaging techniques to assess intracranial atherosclerosis, such as computed tomographic angiography or magnetic resonance angiography, provide information on the degree of narrowing of the vascular lumen. Most classification schemes for ischemic stroke etiology require plaque to cause ≥ 50% stenosis for a given stroke to be attributable to large artery atherosclerosis [19]. Magnetic resonance imaging (MRI) studies of the extracranial carotid arteries, however, suggest that many atherosclerotic plaques have high-risk features despite the absence of significant luminal narrowing [20,21]. It is unknown to what extent similar non-stenotic intracranial atherosclerotic plaque may be responsible for a proportion of the approximately 1 in 3 ischemic strokes for which no clear etiology can be determined [22].

Recent investigations have begun to address this problem by leveraging high-resolution, multiplanar MRI to detect high risk abnormalities of the intracranial vessel walls. Previous studies in both the coronary and extracranial carotid arteries have shown that abnormal plaque enhancement after the administration of gadolinium contrast agent is a marker of inflammation, neovascularity, and plaque instability [23,24]. For this reason, plaque enhancement has been recently studied as a potential high-risk plaque feature in the intracranial circulation. Plaque enhancement is a particularly attractive MRI biomarker because it can be rapidly detected, qualitatively assessed, and does not require significant image postprocessing to analyze.

A systematic review and meta-analysis to evaluate the association between abnormal plaque enhancement on high-resolution MRI and acute ischemic stroke has been performed [25]. The results indicate that intracranial plaque enhancement on high-resolution vessel wall MRI is strongly associated with ischemic stroke. It was concluded that evaluation of plaque enhancement on MRI may be a useful test to improve diagnostic accuracy in patients with ischemic strokes of undetermined etiology [24].

Although MRI remains valuable in the investigation and management of ischemic stroke, Watts et al. [26] identified certain stroke syndromes that are more commonly associated with diffusion-weighted imaging (DWI)-negative MRI. This case series identifies 16 cases of DWI-negative stroke, constituting 2.3% of ischemic stroke patients who had MRI. These amount to be about 20,000 of the annual 800,000 new ischemic strokes. They classified almost all cases as either posterior circulation or lacunar stroke, with isolated internuclear ophthalmoplegia and ataxic hemiparesis being the most common syndromes. DWI-negative stroke is likely due to poor image quality rather the lack of diffusion restriction. It is also possible for small lesions to rapidly and temporarily reverse leading to the perception of DWI-negative stroke [27].

One of the minimally-invasive diagnosis of the cerebral ischemia is the gadolinium enhanced MRI imaging [26]. It is mainly used in patients to detect brain tissue damaged by an ischemic stroke and brain hemorrhage. Cerebral angiogram is also used to provide detailed view of the arteries in the brain and the neck

[28]. In majority of cases, CTA and MRI imaging allow sufficient information without the need for cerebral angiogram. In some cases, however, an angiogram is requested due to lack of sufficient information with MRI. These techniques are expensive and, in the case of angiography, include risk of an invasive procedure.

In the absence of blood-brain barrier (BBB) disruption, gadolinium contrast given during MRI remains in the intravascular compartment and does not enter brain parenchyma or cerebrospinal fluid (CSF). When gadolinium leaks into brain parenchyma, it can be detected with dynamic susceptibility contrast imaging [29]. Disruption of the BBB can occur in acute [30] cerebral ischemia and gadolinium can leak into the CSF. Like BBB disruption, the integrity of blood-ocular barrier (BOB) can be affected by vascular disease [31]. Gadolinium leaks into ocular structures (GLOS) has been reported in the setting of central retinal artery occlusion [32] and kidney disease [33], and may therefore be a marker for disruption of the BOB.

2.2 New Concept

2.2.1 Background

Aqueous humor is a clear fluid (98% water) that fills and helps form the anterior and posterior chambers of the eye. Active secretion is thought to be the major contributor to aqueous formation, responsible for approximately 80% to 90% of the total aqueous humor formation. Active transport takes place through selective trans-cellular movement of anions, cations, and other molecules across a concentration gradient in blood-aqueous barrier. The rate of aqueous humor turnover is estimated to be 1-1.5% of the anterior chamber volume per minute which is 2.4 ± 0.6 µl/min (mean ± SD, daytime measurements in adults aged 20-83 years) [34].

The vitreous chamber volume is more than an order of magnitude larger than aqueous chamber. It is surrounded by and attached to the retina and lens of the eye. It is virtually acellular, highly hydrated extracellular gel matrix, composed of approximately 99% water [35]. The vitreous does not undergo a regular formation and drainage process like the aqueous chamber. Instead, it stays permanently in the vitreous body of the eye [36].

Hitomi et al. [37] serendipitously noted GLOS as a common occurance in patients with acute stroke, prompting them to conduct a retrospective study to determine prevalence and assess relationship to clinical and radiographic findings. They studied the frequency and nature of their findings in 167 patients. At 2 hours after administration of the contrast, GLOS was more common in the aqueous chamber alone, occurring in 67% of patients, compared to the vitreous chamber alone, seen in 6% of patients (Fig. 1(A)). GLOS occurred in both chambers in 27% of patients. At 24 hours, GLOS was present in 75% patients, always involving the vitreous chamber, but also affecting the aqueous chamber in 10% of cases, Fig. 1(B). Patients with rapid diffusion of GLOS, defined as GLOS involving both chambers at 2 hours, had larger infarcts ($p = 0.022$) and a higher degree of BBB permeability ($p = 0.025$), Fig. 1(C). They concluded that GLOS is common in patients with acute stroke and delayed GLOS was a marker for chronic vascular disease. Fig. 1(C) shows an example of a rapid diffuse GLOS. The MRI image shows a high concentration of Gadolinium-DTPA in the eye chamber cavity of patients with disrupted BOB, when injected with 0.1 mmol/kg of body weight. One of the key findings was that BOB disruption, when detected with GLOS often follows a temporal pattern with GLOS in the aqueous fluid within minutes to hours, followed by GLOS in the vitreous fluid on the order of hours to days. It is expected that other mechanisms in addition to the active transport are responsible for the transport of Gadolinium to the aqueous humor when blood-aqueous barrier (BAB) is disrupted. The BOB is composed primarily of a blood-retinal barrier (BRB) and BAB. This phenomenon has also been recently observed by a German group [38-40].

2.2.2 Proposed Contrast Agent

It is preferable to use commercially available sodium fluorescein rather than gadolinium due to its high absorption coefficient in the visible spectrum. Although commonly referred to as fluorescein, the dye used for fluorescein angiography is sodium fluorescein, the water-soluble salt ($C_{20}\ H_{10}\ Na_2\ O_5$) [41]. Fluorescein angiography is performed by injecting sodium fluorescein dye as a bolus into a peripheral vein. Upon entering the circulation, approximately 80% of the dye molecules bind to plasma proteins, which

significantly reduces fluorescence because the free electrons that form this chemical bond are subsequently unavailable for excitation. The remaining unbound or free fluorescein molecules fluoresce in the green light range when excited with light of the appropriate wavelength. With a molecular weight of 376, fluorescein diffuses freely out of all capillaries except those of the central nervous system, including the retina.

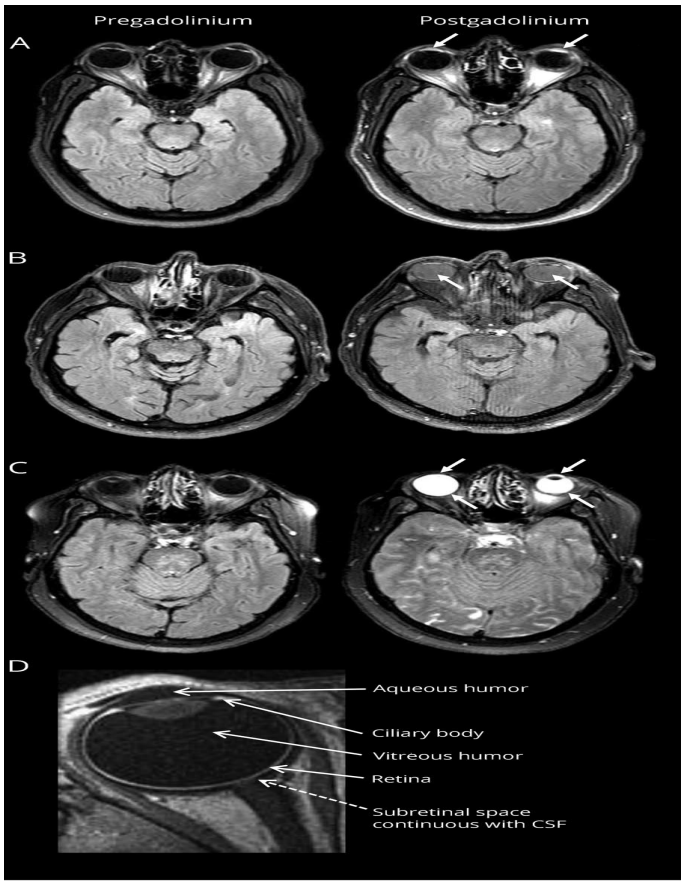

Figure 1: Pre- and post-gadolinium fluid-attenuated inversion recovery MRI images are shown for patients demonstrating GLOS of the aqueous chamber (A, indicated by arrows), the vitreous chamber (B, indicated by arrows), and both (C, indicated by arrows). C) Example of rapid diffuse GLOS. D) Ocular structures (Reproduced with permission) [37]

The dye is metabolized by the kidneys and is eliminated through the urine within 24 to 36 hours of administration [41]. In a typical fluorescein angiography, about 12 s after the injection, the dye appears in the arteries of the retina. Over a 2 to 5 s period, the dye travels through the very small vessels and fills the veins. Ten minutes after injection, the dye mostly evacuated from the eye [42]. In the case of stroke as discussed above, it takes longer (1-2 hour) to observe the contrast agent in the eye that has diffused to the aqueous chamber through the BBB and (BAB) rupture and consequently there is no contrast agent left in the vascular system of the eye before the sodium fluorescein appears in the aqueous chamber resulting from the barrier rupture. This dynamic provides us with an isolated signal associated with possible ischemic stroke.

The rationale is that the detection of sodium fluorescein in the eye can be made much more readily and inexpensively than with an MRI of the brain. This can also be used to pre-screen patients that may require an MRI and this technology can be potentially used at outpatient centers. Based on the foregoing observations, we postulate that it is plausible to detect the acute stroke and transient ischemic attack (TIA) through the detection of contrast agent in the eye by i.v. injection. It is expected that there will not be any contrast agent in the eye for TIA cases due to the lack of BBB disruption.

The fluorescein angiography camera prices are affordable, cost effective, and can be used to detect acute stroke in patients and TIA to reduce the costs of MRI imaging. This method also detects acute stroke when there is false negative MRI which is 2-3% [24]. The clinician can then decide whether the acute stroke patient should undergo MRI imaging for further therapy related diagnosis.

2.2.3 Complications and Adverse Reactions of Fluorescein Sodium

A retrospective review of all adverse reactions to intravenous sodium fluorescein in patients undergoing fluorescein angiography between June 1998 and June 2004 was undertaken [43]. The total number of fluorescein angiograms performed and the number of patients with adverse reactions were identified from the photographic department database and the fluorescein adverse reaction register at the Lions Eye Institute. A total of 11,898 fluorescein angiograms were performed during the study period in Australia. There were 132 adverse reactions recorded with the commonest adverse reactions being nausea and vomiting. There were no serious adverse reactions or deaths recorded. There was a statistically significant difference in the incidence of adverse reactions between sodium fluorescein used from two manufacturers. The tests used a dose of 5 ml sodium fluorescein 10% which is about 500 mg of sodium fluorescein. In recent years, the need to perform fluorescein angiography in elderly people as treatment modalities for age-related macular degeneration (AMD) have increased. In a retrospective study of 358 patients, adverse reactions to fluorescein in relation to advanced age and hypertension were evaluated. It was concluded that a statistically significant correlation between adverse reactions and age or hypertension did not exist [44]. These mild reactions typically occur 30-60 s after injection and last for about 1 to 2 minutes. Fortunately, they seldom compromise the diagnostic quality of the angiogram. The incidence of nausea and vomiting seems to be related to the volume of dye and rate of injection. A relatively slow rate of injection often reduces or eliminates this type of reaction but can adversely affect image quality and alter arm-to-retina circulation times. Premedication with promethazine hydrochloride or prochlorperazine may prevent or lessen the severity of nausea and vomiting in patients with a history of previous reactions to fluorescein but is rarely needed and one study noted a higher frequency of these reactions in patients that had been premedicated. Some patients report a strong taste sensation or hypersalivation following injection of fluorescein [45].

2.2.4 Level of Detection Estimate Using Fluorescein Angiography

The estimate for level of detection will be made based on the value of the sodium fluorescein injection. We suggest 500 mg of fluorescein which is a nominal value for fluorescein angiography for diagnosis and treatment of retinal disorders for human [42]. This amount is present in 5 ml of 10% sodium fluorescein solution. We anticipate that the sodium fluorescein can diffuse into the aqueous chamber when the BBB and blood aqueous barrier is disrupted. The concentration of sodium fluorescein in the patient vascular system is 5 ml/5000 ml = 10^{-1}% with a human body mass of 70 kg, assuming 5 liters of blood volume. A fraction of the contrast agent from the vascular system will flow through the BBB and BAB due to the stroke. A very small fraction of 10^{-3}%, will translate into the contrast agent concentration of 10^{-4}% in the aqueous chamber.

Sodium fluorescein absorbs blue light, with peak excitation occurring at wavelengths between 465-490 nm. The resulting fluorescence occurs at the yellow-green wavelengths of 520 to 535 nm [46]. The peak molar extinction coefficient is 92,300 $cm^{-1}M^{-1}$ at 535 nm. In broad-spectrum illumination, diluted sodium fluorescein appears bright yellow-green in color. When illuminated with blue light, the yellow-green color intensifies dramatically. Fluorescence is detectable in concentrations between 10^{-1}% and 10^{-7}% due to its very high extinction coefficient [46]. The visible light absorption of the lens (content of lens is mostly water) at this wavelength is almost close to zero (Fig. 2) which results in zero transmission loss going through the lens [47]. The minimum concentration (detection limit) of the sodium fluorescein inside the eye cavity can be calculated using Beer's law from numbers above and light path length (light travels 23 mm [48] through the lens to reach the retina before returning to the camera). This detection limit is much lower than the 10^{-4}% in the aqueous

chamber. The resulting fluorescence signal generated within the aqueous chamber reaches the camera. The camera can be the same as used for the eye fluorescein angiography.

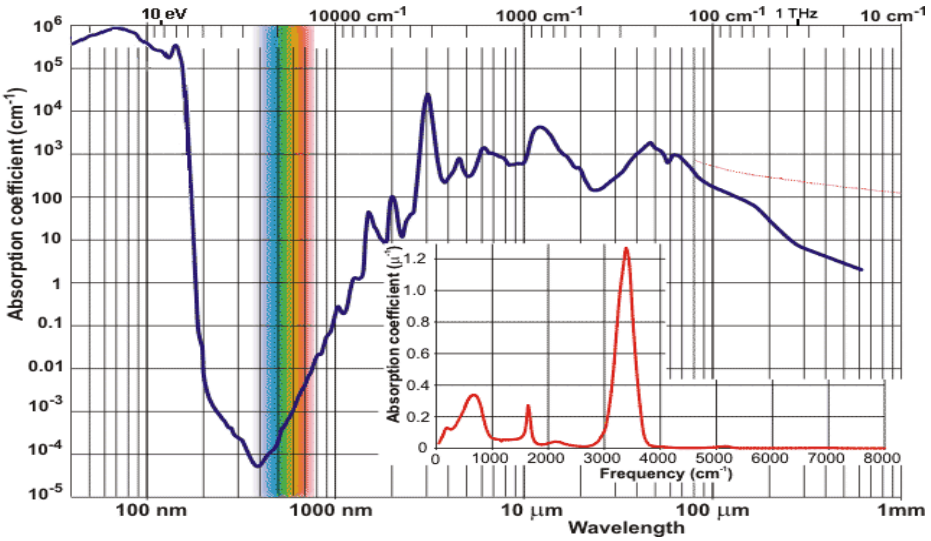

Figure 2: Spectra of absorption coefficient of liquid water

3 Discussion

The proposed approach needs to be tested with an animal model. The majority of stroke experiments are carried out in small animals (e.g., mice, rats, rabbits). The use of small animals presents clear advantages-lower cost and greater acceptability from an ethical perspective-compared to larger animals. Mice are the most commonly used animals in stroke studies for many reasons given the following advantages: 1) Cerebral vasculature and physiology of the mice is similar to that of humans; 2) Moderate body size allows easy monitoring of physiologic parameters; 3) Small brain size is well suited to fixation procedures (e.g., *in vivo* freeze trapping for biochemical analysis). There is a relative homogeneity within strains; and 5) It is relatively easy to conduct reproducible studies [11].

It is important to note that mouse stroke model may not be a good model for therapeutic clinical trials. As an example, the enthusiasm of the preclinical success of induced pluripotent stem cell-derived neural stem cells (iNSCs) therapy has been tempered with the hundreds of failed clinical trials of therapeutics previously developed in rodents and a need for a deeper understanding of the underlying recovery mechanisms [49]. A pig stroke model utilizing translational approaches. However, showed that iNSC cell therapy leads to significant tissue recovery and cell replacement. The mouse stroke model has been successfully used to model patient ischemic stroke (transient, as well as acute) for diagnostic purposes.

No animal model can recapitulate all aspects of human stroke because ischemic stroke in humans is a heterogeneous disorder with complex pathophysiology [11]. The transient or permanent middle cerebral artery occlusion (MCAo) model is one of the models that most closely simulate human ischemic stroke [11]. Furthermore, this model is characterized by reliable and well-reproducible infarcts. Therefore, the MCAo model has been used in majority of studies that address pathophysiological processes or neuroprotective agents [11]. The middle cerebral artery (MCA) and its branches are the cerebral vessels that are most often affected in human ischemic stroke, accounting for approximately 70% of infarcts [50]. The technique that occludes this artery reproduces conditions similar to human ischemic stroke and hence can be used in animal model tests.

To establish a model of cerebral ischemia characterized by both a robust BBB disruption and a sizable infarct volume, mice should be subjected to a distal MCAo (dMCAo). The greatest infarct volume can be produced by 120 min of ischemic time induced by dMCAo + ipsilateral [51]. Compared to other protocols,

this method also will produce the greatest unilateral increase in BBB permeability. Nominal quantity of sodium fluorescein should be injected after the MCAo procedure is complete.

4 Conclusion

We propose a new method to detect acute stroke and TIA through the detection of sodium fluorescein contrast agent in the eye by i.v. injection. The agent is safe and is used routinely in eye fluorescein angiography. The fluorescein angiography camera prices are affordable and this method avoids some of the shortcomings of MRI imaging and angiogram like cost, X-ray exposure, and facility availability in small communities. This method with the angiography camera embedded in the cell phone [52] can detect cerebral ischemia in real-time with reduced cost of diagnosis. This can have far-reaching impact on stroke management and associated costs. Ultimately, the device should be portable and simple to use by a medical technician in medical office, emergency outfits or even in ambulances.

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