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Molecular Hydrogen Therapy Enhances Immune Markers in Treg, Plasma, Tr1 Cells, and KLRG1 Expression on Tc Cells: A Case of Acute SDH With Midline Shift and Uncal Herniation Post-decompressive Craniectomy

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Abstract

Background/Aim: Subdural hematomas (SDH), often caused by head trauma, are serious with high mortality and long-term complications. Studies show that molecular hydrogen has neuroprotective effects, such as reducing oxidative stress, inflammation, and cell death. It may also protect mitochondria, support cell function, and regulate immune responses, making it a promising new treatment option for SDH. However, more research is needed to confirm its effectiveness and create treatment guidelines.

Case Report: We present a 24-year-old man with SDH, along with a right-sided midline shift, uncal herniation, and dilated left pupil. Conventional treatments—craniectomy, hyperbaric oxygen, therapeutic hypothermia, and stem cell therapy—were essential for stabilizing his condition. In addition, we administered hydrogen capsules as a novel adjunct therapy, beginning daily treatment immediately upon admission. While recovery was primarily due to

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standard interventions, hydrogen therapy appeared to enhance immune markers, particularly Treg and plasma cells, with no adverse effects. This case indicates that hydrogen therapy may serve as a beneficial addition to established SDH management methods.

Conclusion: This case suggests that molecular hydrogen therapy may be a helpful adjunct treatment for SDH with midline shift. Conventional therapies, including craniectomy, hyperbaric oxygen, therapeutic hypothermia, and stem cell therapy, were vital to the patient's recovery, but hydrogen therapy may have contributed by modulating immune responses, particularly Treg and plasma cell activity. While these findings are encouraging, further research is necessary to confirm hydrogen therapy's benefits and its role alongside traditional neurocritical care treatments.

Keywords: Hydrogen therapy, SDH with midline shift, T regulatory cells, plasma cells, case report.

Introduction

Most acute subdural hemorrhages are caused by traumatic head injuries and often have devastating outcomes, with high mortality and significant long-term complications among survivors (1). In recent decades, patient outcomes of traumatic brain injury (TBI) have improved somewhat due to advancements in emergency response, faster and more accurate computed tomography (CT) scanning, and critical care improvements (2, 3). However, outcomes of acute subdural hematoma (ASDH) have not seen the same level of improvement. Mortality rates for ASDH range from 50% to 90%, especially higher when anticoagulant therapies are involved (4). ASDH is frequently accompanied by other brain and body injuries, further increasing the risk of morbidity and mortality. With an aging population, these outcomes may worsen in the future (5).

Recent research has shown that hydrogen therapy functions through anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms. Molecular hydrogen (H_2) easily crosses cell membranes, helping to reduce excessive reactive oxygen species (ROS) and modulate various cellular signaling pathways (6). This action protects mitochondria, enhances adenosine triphosphate (ATP) production, and regulates cell death processes, including apoptosis and autophagy (7, 8). Hydrogen therapy is particularly promising for treating brain injuries, as it may reduce oxidative stress and alleviate inflammatory responses that can worsen damage after trauma (9). Studies indicate that this therapy could facilitate recovery and improve outcomes for patients with TBI, including those with complications, such as subdural hematomas and cerebral edema (8). Additionally, the potential of hydrogen therapy in neuroprotection makes it a compelling subject for future research in brain injury management (10).

We present the case of a 24-year-old man with a subdural hematoma (SDH), accompanied by a right-sided midline shift, uncal herniation, and a dilated left pupil. The patient's neurological condition gradually improved with a combination of surgical intervention, pharmacological treatments, and supportive therapies, including hyperbaric oxygen, therapeutic hypothermia, endogenous stem cell therapy, and adjunctive hydrogen therapy. We also analyzed immune cell phenotypes before and after treatment to characterize immune responses and evaluate the impact of hydrogen-assisted therapy. This study was approved by the Institutional Review Board (IRB) of Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, in accordance with relevant guidelines (IRB No. B202105106, approval date: January 16, 2024). Written informed consent was obtained from all participants (Consent No. B202105106-36). The study adhered to the ethical standards of the institution and complied with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Case Report

We present the case of a 24-year-old male student from the National Defense Medical Center who was diagnosed with SDH after a motorcycle accident in Penghu. He exhibited a right-sided midline shift, uncal herniation, and a dilated left pupil. Following the accident, he received extensive treatment (Figure 1) to stabilize his condition. The patient was initially admitted to the Penghu Branch of Tri-Service General Hospital on June 9, 2024, with multiple injuries, including fractures of the basal skull, left temporal bone, and left zygomatic arch, as well as a left maxillary sinus fracture. He also suffered a brain contusion with an acute SDH, resulting in a right midline shift (Figure 2A), uncal herniation, and left pupil dilation. A neurological examination revealed a Glasgow Coma Scale (GCS) score of E1M1VT, with bilateral Babinski and Hoffmann signs, indicating significant neurological impairment.

In response to these severe injuries, an emergency decompressive craniectomy was performed on the left frontal, temporal, and parietal (F-T-P) regions, The procedure included the removal of the subdural hematoma and the placement of an intracranial pressure monitor. Following this initial treatment, the patient was transferred to Neihu Tri-Service General Hospital on June 11, 2024, for further management and evaluation. Management of cerebral perfusion pressure (CPP) and target temperature management (TTM) was initiated, with a target temperature set at 36°C. An ophthalmology consultation was requested to assess the orbital fracture and ecchymosis, which revealed a tripod fracture with possible traumatic optic neuropathy. The prognosis was discussed with the family, indicating a poor outcome. Nephrology was also consulted for the management of hypernatremia and polyuria, with central diabetes insipidus (DI) suspected. The patient received free water supplementation and desmopressin (DDAVP) for treatment. In addition, hyperbaric oxygen therapy (HBOT), therapeutic hypothermia, and stem cell therapy were arranged for the severe brain injury, and an otolaryngology specialist performed bilateral myringotomy.

On June 26, after the reduction of brain swelling, a CT scan (Figure 2B) revealed bilateral subdural effusion with effacement of the sulcus on the right side, likely due to intracranial hypotension from cerebrospinal fluid (CSF) leakage. On June 28, magnetic resonance (MR) myelography confirmed CSF leakage from the spine (Figure 2C). An anesthesiologist subsequently performed an epidural blood patch. However, a CT scan (Figure 2D) on July 6 showed persistent subdural effusion. To address this, a CT-guided epidural blood patch was performed on July 9 by a radiologist. A follow-up CT scan (Figure 2E) on July 19 demonstrated improvement in the subdural effusion, and the patient's neurological status also improved, with a GCS score of 8T. Between July 19 and August 19, the patient exhibited progressive improvements in neurological function. By July 26, he was able to initiate verbal communication. On August 10, he regained the ability to write, and by August 16, he could stand with assistance.

Given the patient's stable condition, a cranioplasty was planned and performed on August 19. Before the procedure, a 3D-printed model (Figure 3) was created to enhance the surgical process. Post-operatively, the patient showed significant improvement. By August 31, the GCS score had risen to E4M6VT, indicating marked recovery. In addition to the conventional therapies, including craniectomy, hyperbaric oxygen therapy, therapeutic hypothermia, and stem cell therapy, we introduced a novel treatment with hydrogen capsules. Although hydrogen therapy has been explored in various clinical cases and many articles have been published, it is not yet a standard treatment for SDH and remains an unconventional option for some specialists. We administered hydrogen capsules to the patient twice daily (BID) starting on June 12. Following the initiation of hydrogen therapy, we assessed the patient's immune regulatory factors, including Treg cells, plasma cells, and KLRG1 expression on Tc cells, on June 17. After two months of therapy, we re-evaluated the data on August 5 and September 30, ultimately observing significant improvements in immune factors.

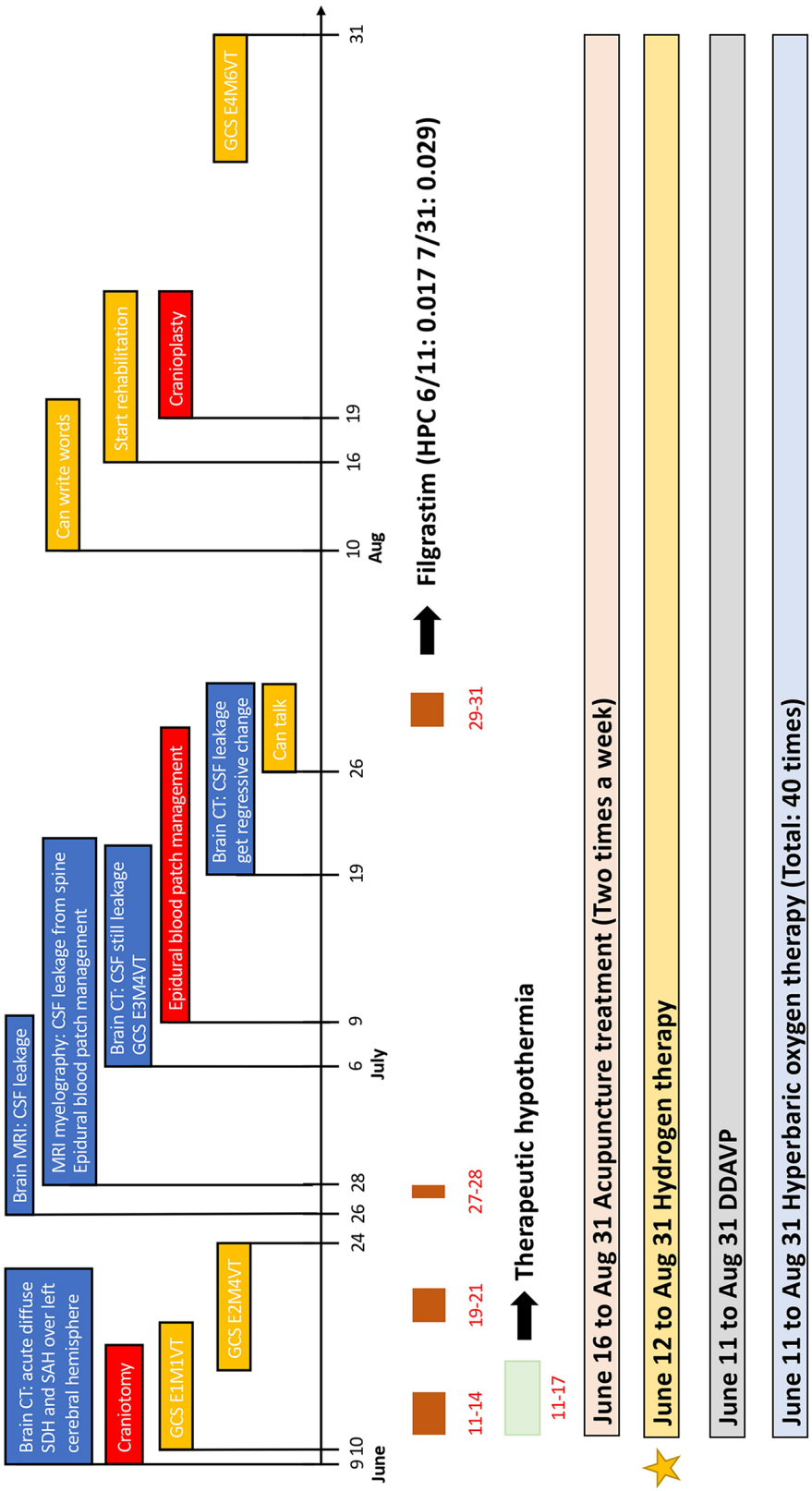

Figure 1. *Disease progression and clinical treatment course. The timeline features three distinct colors. The blue line represents imaging examinations and their corresponding findings. The red line denotes surgical interventions performed during the treatment course. The yellow line illustrates the patient's neurological condition. Additionally, the various colors below the timeline indicate the patient's medication regimen.*

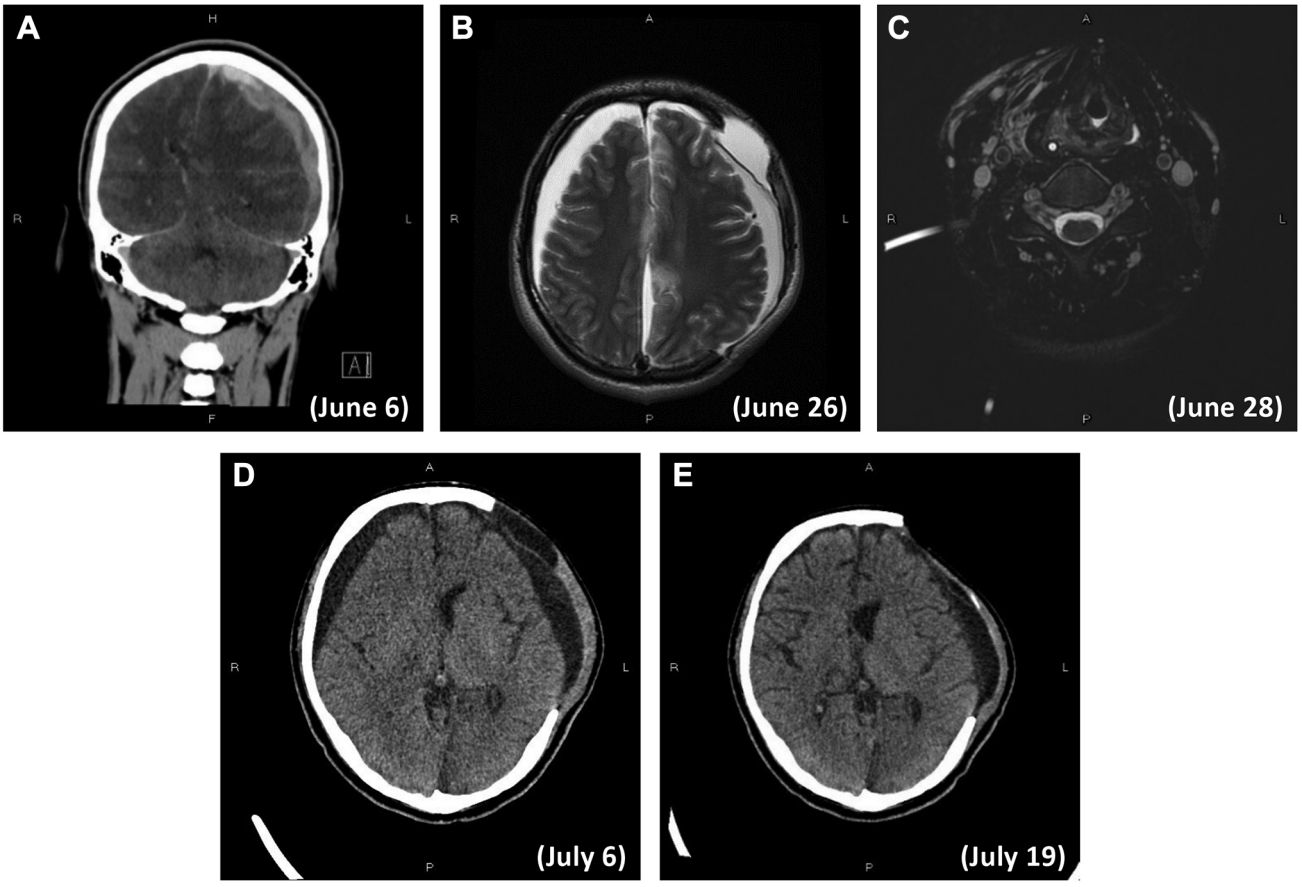

Figure 2. *Progressive imaging of acute subdural hematoma (SDH) and post-traumatic cerebrospinal fluid (CSF) leak-induced subdural effusion with intervention outcomes. (A) Computed tomography (CT) scan taken on June 6, 2024, reveals a significant right-sided midline shift resulting from acute subdural hematoma. The scan also indicates uncal herniation and compression of the right lateral ventricle. (B) Magnetic resonance imaging (MRI) on June 26, 2024, displays bilateral subdural effusion with sulcal effacement on the right side, likely attributable to CSF leakage. This effusion aligns with post-traumatic intracranial hypotension. (C) MRI myelography on June 28, 2024, confirms the presence of CSF leakage, which contributes to the subdural effusion observed in earlier scans. (D) CT scan on July 6, 2024, shows persistent subdural effusion despite interventions, with only minimal reduction in effusion size. (E) Follow-up CT scan on July 19, 2024, demonstrates substantial improvement in the subdural effusion, resolution of the midline shift, and recovery of normal ventricle size.*

Despite the high mortality rates and significant long-term complications associated with SDH, along with the severity of midline shift and a low GCS score for this patient, our combined therapy—which included hyperbaric oxygen therapy, therapeutic hypothermia, stem cell therapy, and hydrogen therapy—resulted in substantial progress in the patient's condition. Notably, the GCS score improved from E1M1VT to E4M6VT, and the patient is now able to speak clearly and write accurately.

Discussion

This case highlights the urgent need for innovative therapeutic strategies in managing TBI, particularly when complicated by SDH with significant midline shift and uncal herniation. In addition to employing aggressive conventional treatments, such as decompressive craniectomy, hyperbaric oxygen therapy, therapeutic hypothermia, and stem cell therapy, we introduced hydrogen therapy as an adjunctive treatment. This approach showed promising potential by

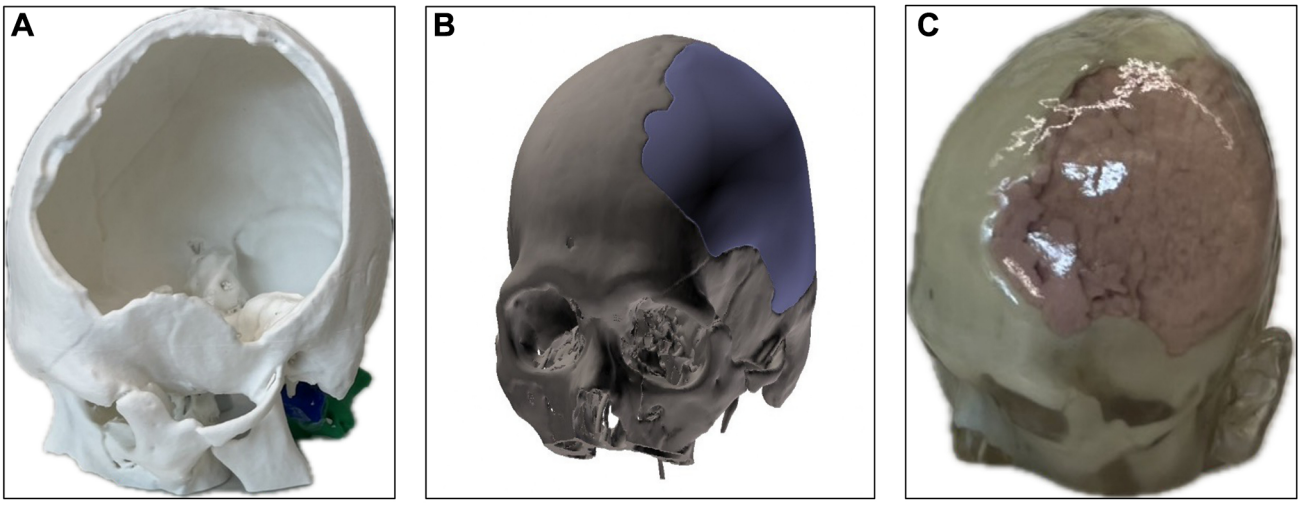

Figure 3. *Enhancing surgical preparation with 3D printed anatomical models. Images illustrate products created using 3D printing technology. These models provide physicians with a detailed understanding of anatomical structures prior to surgery, facilitating a smoother surgical process. Additionally, they serve as valuable resources for clinical teaching and education.*

modulating immune responses and improving clinical outcomes, thereby providing valuable insights for clinicians in this area (11).

The pathological changes that occur following SDH are generally classified into early brain injury (EBI) and delayed brain injury (DBI). EBI is characterized by increased intracranial pressure (ICP), decreased cerebral blood flow, and global ischemia, resulting in disruption of the blood-brain barrier, inflammation, brain edema, and neuronal death (12). DBI is associated with focal neurological deficits and cognitive impairments, making it a significant contributor to mortality and morbidity following SDH. Immune cells, particularly astrocytes and microglia, play critical roles in this damage (13). After a brain injury, astrocytes release S100B, which elevates ROS levels in neurons and glial cells. This increase further activates phosphorylated c-Jun N-terminal kinase (p-JNK) in neurons, leading to apoptosis. A study conducted with a rat model of SDH found that inhalation of hydrogen gas reduced DBI by attenuating EBI and decreasing levels of S100 calcium-binding protein B (S100B) and p-JNK, both of which are key contributors to the progression of EBI (14).

Microglia, the primary immune cells of the central nervous system (CNS), play a crucial role in the immune response to injury. Upon activation, microglia can adopt one of two states: classical (M1) or alternative (M2). M1 microglia are activated by factors such as interferon-gamma (IFN-γ) and lipopolysaccharide (LPS), leading to pro-inflammatory responses and contributing to neuroinflammation through the production of ROS and nitric oxide (NO). This inflammatory state can worsen brain damage. In contrast, M2 microglia, which are activated by anti-inflammatory cytokines like interleukin-4 (IL-4) and interleukin-13 (IL-13), secrete factors that promote tissue repair, including neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). M2 microglia also aid in debris clearance and support neuronal survival, facilitating recovery after injury (15, 16). In addition to astrocytes and microglia cells, plasma cells also play an important role in SDH (17). Plasma cells, which represent the final stage of B cell differentiation, are primarily responsible for secreting antibodies (18). These antibodies are essential for recognizing and neutralizing pathogens during immune responses. However, in the context of acute brain

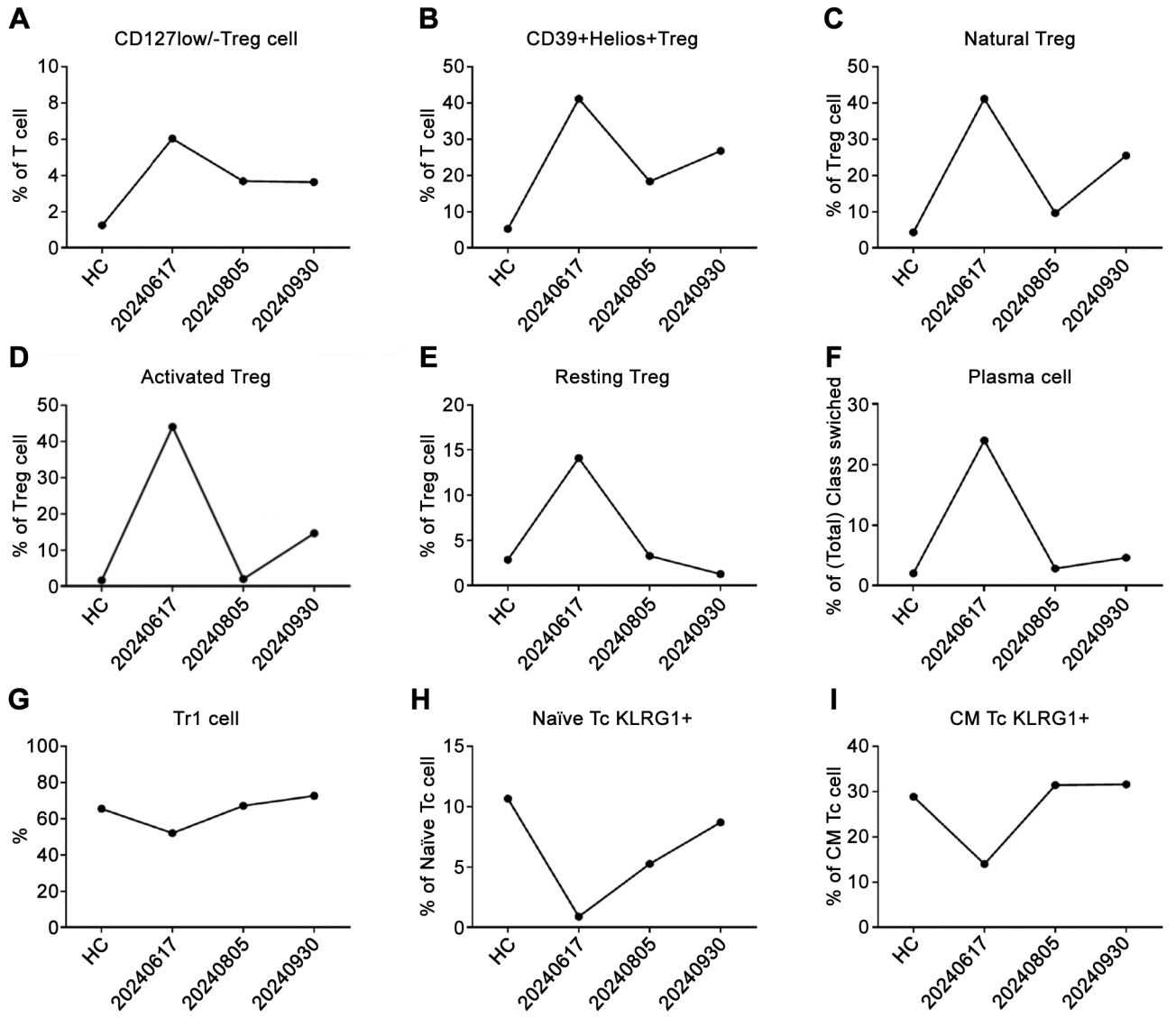

Figure 4. *Immunophenotypic profiles of Treg cells, plasma cells, and cytotoxic T cells were analyzed at various time points. (A-F) the percentages of different Treg subsets, including CD127low/- Treg cells (A), CD39+ Helios+ Treg cells (B), natural Treg cells (C), activated Treg cells (D), resting Treg cells (E), and plasma cells (F) following the interventions. (G-I) Panels illustrate the changes in Tr1 cells (G), naive Tc cells expressing KLRG1 (H), and central memory Tc cells expressing KLRG1 (I) over the same timeline. The abbreviations used are as follows: HC: healthy control; CD127low/-Treg: CD3+ CD4+ CD25high CD127low/-; CD39+Helios+Treg: CD3+ CD4+ CD25high CD39+ Helios+; natural Treg: CD3+ CD4+ CD25high FoxP3+ Helios+; activated Treg: CD3+ CD4+ CD25high FOXP3high CD45RA-; resting Treg: CD3+ CD4+ CD25high FoxP3low CD45RA+; plasma cells: CD19+ CD38high IgD-; Tc: cytotoxic T cells (CD3+CD8+); naive Tc: CD3+ CD8+ CCR7+ CD45RA+; central memory Tc: CD3+ CD8+ CCR7+ CD45RA-.*

injuries, such as TBI, SDH, or stroke, the role of plasma cells may extend beyond protective immunity, potentially contributing to adverse outcomes (19).

Regulatory T cells (Tregs) play a critical role in modulating the immune responses of astrocytes and microglia. These cells secrete anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), which suppress M1 activation and promote the transition to the M2 phenotype, facilitating inflammation resolution and tissue repair. Tregs

also interact directly with microglia through cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) receptors, inhibiting pro-inflammatory responses and supporting M2 activation. Additionally, Tregs influence microglial metabolism by shifting it from a glycolytic pathway—associated with M1 activation—to oxidative phosphorylation, thereby sustaining M2 microglial function and survival (15). In addition to regulating astrocytes and microglia, Treg cells also play a significant role in modulating plasma cells. Treg cells regulate plasma cells primarily through both direct and indirect mechanisms. Indirectly, Tregs suppress T helper (Th) cells and follicular helper T cells (Tfh), which are crucial for B cell activation and plasma cell differentiation. They achieve this suppression by using molecules like CTLA-4 to modulate antigen-presenting cells (APCs) and by secreting anti-inflammatory cytokines, such as IL-10 and TGF-β. Furthermore, emerging studies suggest that Tregs can directly suppress plasma cells by limiting their proliferation and antibody production, although the precise pathways involved are still under investigation.

Recognizing the crucial role of the immune system in the progression of intracerebral hemorrhage (ICH) and SDH, we conducted immunophenotyping analyses to assess changes in immune cell populations before and after treatment, evaluating the efficacy of hydrogen-assisted therapy (Figure 4). The blood sample collected on June 17 likely reflects the early inflammatory response characteristic of the initial stages of ICH and SDH, occurring prior to the full therapeutic effects of hydrogen therapy. Notably, the introduction of hydrogen therapy was associated with a significant increase in various subsets of Tregs on June 17, followed by a decline observed on August 5 and September 30. This dynamic change suggests that hydrogen therapy may play a significant role in modulating Treg activity. As the patient's condition stabilized, the initially heightened involvement of Tregs in suppressing inflammation appeared to diminish, indicating a return to immune homeostasis. These findings underscore the role of Tregs in maintaining immune equilibrium and promoting tissue repair, consistent with the patient's transition from the acute phase to recovery. This trend aligns with the dynamics observed in plasma cells, where an initial increase was followed by a subsequent decrease, reflecting the immune regulatory influence of Tregs. The early rise in Tregs may have played a critical role in suppressing the heightened activity of plasma cells, which are responsible for producing antibodies that could exacerbate inflammation and worsen brain injury. This interplay between Tregs and plasma cells indicates that Tregs were actively involved in modulating the immune response, preventing excessive antibody-mediated inflammation during the patient's recovery phase (20).

Hydrogen therapy, recognized for its ability to regulate immune function through pathways, such as nuclear factor erythroid 2-related factor 2 (NRF2), may have played a crucial role in the observed changes (21). By modulating oxidative stress and inflammation, hydrogen therapy likely facilitated the initial increase in Treg activity, which helped control plasma cell proliferation, antibody secretion, and the activity of astrocytes and microglia, thereby preventing further brain damage. The subsequent stabilization of Treg levels and suppression of plasma cells suggest that immune modulation persisted throughout the later stages of recovery, with Tregs maintaining a balanced immune response as the patient transitioned from the acute phase to recovery. This pattern underscores the dynamic nature of immune regulation in response to treatment, highlighting the role of Tregs in controlling inflammation and facilitating tissue repair. At the same time, Tregs suppress the activity of plasma cells, astrocytes, and microglia to mitigate adverse outcomes associated with SDH.

In contrast to the Treg cells, Tr1 cells, which lack forkhead box P3 (FoxP3) expression, exhibited different trend. Following the introduction of hydrogen therapy, Tr1 cells showed a decline, suggesting a possible shift in the immune regulation mechanism. This opposing pattern between Tregs and Tr1 cells underscores the complexity of immune modulation during the recovery phase. Additionally, we observed that the expression patterns of

naïve KLRG1+ Tc cells and central memory KLRG1+ Tc cells initially decreased during the injury but gradually returned to normal levels. We hypothesize that this may be related to hydrogen therapy; however, further confirmation is necessary (22). The dynamic changes observed in immune cell populations, particularly the increase in Tregs and the fluctuations in B cells, highlight the importance of incorporating immunophenotyping into the clinical assessment of TBI. As a novel adjunctive treatment, hydrogen therapy has shown promise in modulating immune responses and facilitating recovery. Future research should aim to deepen our understanding of the roles of immune factors in TBI recovery to refine therapeutic strategies and optimize patient outcomes.

Conclusion

In conclusion, this case study underscores the potential effectiveness of hydrogen-assisted therapy in managing severe TBI complicated by SDH, including cases with acute SDH, midline shift, and uncal herniation. The observed therapeutic effects, particularly the modulation of Treg cells, plasma cells, Tr1 cells, and KLRG1 expression on Tc cells, highlight the promise of hydrogen therapy as an adjunctive treatment in neurocritical care. However, the limited sample size of this study indicates the need for further research involving larger cohorts and longer follow-up periods to validate these findings. Future studies should also investigate the relationship between hydrogen therapy and Treg cells and plasma cells to better understand its broader applicability in similar clinical scenarios.

Conflicts of Interest

The Authors declare that there are no conflicts of interest related to this research.

Authors' Contributions

HFH, RYH, JWL, DYH: Writing, review and editing, validation, data curation, conceptualization. YJH, SWL: Writing, review and editing, data curation. TYH, KYW, HCL: Writing, review and editing, conceptualization. FCL: Writing, review and editing, writing original draft, resources, investigation, data curation, conceptualization.

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