Glycosylated IgG antibodies accelerated the recovery of haemorrhagic fever with renal syndrome patients

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Abstract

Haemorrhagic fever with renal syndrome (HFRS) is a fatal disease caused by Hantaan virus (HTNV) infection. Humoral immunity is essential for effective viral clearance, but the glycosylation characteristics of immunoglobulin G (IgG) in HFRS patients is not well known. Peripheral blood mononuclear cells from HFRS patients were obtained for B subset analysis using scRNA-seq and flow cytometry. HTNV specific IgG antibody titers were detected by enzyme linked immunosorbent assay, and IgG glycosylation was analyzed by ultra-performance liquid chromatography. The proportions of the antibody-secreting memory (ASM) B cells and plasmablasts (PB) were significantly expanded among acute HFRS patients. We discovered significantly increased fucosylated IgG and decreased bisecting N-acetylglucosamine during the convalescent phase of HTNV infection. However, positive correlations were observed between ASM subsets and galactosylation/sialylation in the IgG Fc region, and between PB subsets and sialylation. Notably, the glycosylation-related genes were primarily differentially expressed in the ASM and PB subclusters, such as *PRN1* and *PRN2*, which were enriched in the N-glycosylation modifications of proteins through asparagine. Our findings indicated that IgG N-glycosylation may play a crucial role in combating HTNV infection and contributing to clinical recovery, which provided new insights for optimizing glycoengineered therapeutic antibodies.

Keywords: Haemorrhagic fever with renal syndrome (HFRS); Hantaan virus (HTNV); B cell; Immunoglobulin G (IgG); Glycosylation; Inflammation

Introduction

The causative agent of Haemorrhagic fever with renal syndrome (HFRS) is Hantaan virus, which belongs to the family *Hantaviridae* and is primarily transmitted by rodents (Zheng et al., 2019). China is the most heavily affected country by HFRS, accounting for >90% of global cases, and a total of 32,462 cases of HFRS were reported in China during 2019–2022, with a fatality rate ranging from 0.1 to 15% (R. Li et al., 2023) (https://www.chinacdc.cn).

Immunoglobulin G (IgG) is one of the best-understood glycoproteins and each constant heavy 2 domain (CH2) of the fragment crystallisable (Fc) region carries a covalently attached biantennary *N*-glycan at the highly conserved asparagine at residue 297 (Trastoy et al., 2018). Functionally, minor alterations in the glycan composition can drastically affect the structural conformation of the Fc region with enormous IgG effector functions (Abu-Raya, Michalski, Sadarangani, & Lavoie, 2020). IgG modulates both pro- and anti-inflammatory activities depending on the moieties of the glycans. For example, sialylation of IgG-Fc reverses a pro-inflammatory process into an anti-inflammatory effect (Pereira et al., 2020). It has been evidenced that progression of dengue virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is regulated by specific IgG glycosylation (Hou et al., 2021; Wang et al., 2017). The human immune response plays a fundamental role in anti-viral infections. Massive and rapidly expanding plasmablast (PB) cells are required for the recovery of HFRS patients (García et al., 2017; Y. Li et al., 2020), however, the increased amount of circulating $CD27^{-}IgD^{-}$ B cells may partially associate with kidney dysfunction (Kerkman et al., 2021). However, there is limited report regarding the regulation of IgG glycosylation in HFRS.

In the present study, we combined scRNA-seq and flow cytometry to reveal the

phenotypes of B cell responses during Hantaan virus (HTNV) infection, and explored the transcriptomic features of reactive B cell subsets. A total of 166 HFRS patients, including 65 paired HFRS samples, were involved to profile the IgG-Fc glycosylation and investigate the potential regulatory role of IgG-Fc glycosylation in HFRS pathogenesis.

Results

B cell compositional characteristics in HFRS patients

To characterize the humoral immune profiles in HFRS patients, we enrolled suspected 166 patients who were admitted to Baoji Central Hospital in Shaanxi Province, China, between October 2019 and January 2022, of whom 65 met the inclusion criteria and were included in the study (Figure 1). We identified a total of eight cell subpopulations, including subsets of CD4 T cells, CD8 T cells, CD14 monocytes, NK cells, B cells, platelet, endothelial progenitor cells, and red blood cells (Figure 2—figure supplement 1A and B). The proportions of these cell types were comparable between acute HFRS patients and healthy controls, with no significant batch effects observed (Figure 2—figure supplement 1C). CD4 T cells (31.4%) were the predominant subset prior to HTNV infection, whereas CD8 T cells (44%) became the dominant population post-infection. Notably, the proportions of NK cells (15.9% to 5.6%, $p<0.001$) and platelets (3.1% to 0.6%, $p<0.01$) significantly decreased, while the level of CD14 monocytes (15.9% to 32.3%, $p<0.05$) nearly doubled. However, the overall proportions of B cells did not exhibit significant changes (Figure 2—figure supplement 1D and E).

To precisely elucidate the role of B cell subsets in anti-HTNV infection, we classified B cells into eight subsets, including antibody-secreting memory B cells (ASM), double-negative B cells (DN), intermediate memory B cells (IM), marginal zone-like B cells (MZB), naïve B cells (naive B), PB, quiescent resting memory B cells (RM), and

exhausted tissue-like memory B cells (TLM) (Figure 2A and B, and Figure 2—figure supplement 1F). We discovered that significant expansion of ASM, PB, and RM cell populations in acute HFRS patients compared to healthy controls, accompanied by contraction of DN, IM, and naïve B cell compartments post-infection (Figure 2C). In healthy controls, naïve B cells (42.87%), DN B cells (26.65%), and TLM B cells (16.13%) constituted the majority of the B cell population. However, ASM (30.96%), naïve B cells (23.85%), and PB cells (11.12%) became the dominant subsets after HTNV infection (Figure 2D and Supplementary file 1).

To validate the accuracy of our single-cell subpopulation analysis, we employed flow cytometry to quantify the B cell subsets in acute and convalescent HFRS patients (Figure 2E). The results demonstrated that ASM (39.48% vs. 24.57%, $p<0.001$) and PB (23.97% vs. 2.61%, $p <0.001$) subsets were significantly amplified in the acute phase compared to the convalescent phase (Figure 2F). Conversely, the number of naive B cells (32.78% vs. 48.92%, $p=0.003$) and CSM cells (18.65% vs. 27.15%, $p=0.008$) rapidly recovered in the convalescent stage (Figure 2F), consistent with the observations at the transcriptome level. No significant differences were observed for other B cell subtypes between the acute and convalescent stages (Figure 2—figure supplement 2). These results clearly exhibited that several B cell subpopulations were effectively activated following HTNV infection.

Dynamic characteristics of the IgG glycome in HFRS patients

HFRS patients were further stratified into three age groups (≤44, 45-59, and ≥60 years). IgG glycosylation was analyzed by ultra-performance liquid chromatography. As shown in Supplementary file 2, no significant differences in IgG glycosylation were observed between age groups, except for galactosylation. Similarly, no gender-based differences in IgG-Fc glycosylation were noted (Supplementary file 3). However, the

levels of IgG with bisecting GlcNAc (15.72% vs. 14.51%, $p<0.001$), galactosylated IgG (74.26% vs. 71.15%, $p<0.001$), and sialylated IgG (22.44% vs. 21.62%, $p<0.001$) were significantly higher in the acute phase compared to the convalescent phase. Conversely, fucosylated IgG levels (94.29% vs. 94.89%, $p<0.001$) were lower in the acute phase (Supplementary file 4), indicating that IgG N-glycosylation plays a critical role in the anti-infection process.

Among the 130 paired blood samples from 65 patients, 38 (29.2%) were negative for HTNV nucleocapsid protein-specific IgG antibodies, while 92 (70.8%) were seropositive. For the 24 patients with both seronegative and seropositive samples, levels of bisecting GlcNAc (16.16% vs. 14.54%, $p<0.001$), galactosylation (74.12% vs. 70.94%, $p=0.006$), and sialylation (22.92% vs. 21.80%, $p=0.008$) were higher in the seronegative period, while that of fucosylation was lower (94.36% vs. 94.74%, $p=0.024$) in this period (Figure 3A). Notably, bisecting GlcNAc and sialylated IgG levels decreased as HTNV-NP-specific antibody titers increased (Figure 3B), highlighting a potential link between antibody levels and IgG-Fc glycosylation.

The IgG glycome in HFRS patients with seroconversion

A 4-fold or greater increase in HTNV-NP-specific antibody titers usually indicates a protective humoral immune response during the acute phase. We observed a significant increase in the Fc fucosylation (94.18% vs. 94.48%, $p=0.044$) and a decrease in bisecting GlcNAc (16.41% vs. 14.71%, $p=0.001$) post the 4-fold antibody titer increase compared to the baseline. However, no significant differences were observed in galactosylation and sialylation (Figure 3C), suggesting that fucosylation and bisecting GlcNAc may play key roles in anti-HTNV infection and recovery.

The potential source of the IgG glycome during HTNV infection

Positive correlations were between the ASM subsets and galactosylation/sialylation in

the antibody Fc region, and between the PB subsets and sialylation (Figure 4A-C). Therefore, we speculated that galactosylated antibodies may primarily originate from the ASM subsets, while sialylated antibodies may derive from the ASM and PB subsets.

To investigate the process of glycosylated antibody production secreted by the ASM and PB subsets, we identified DEGs among the eight B cell subpopulations. Notably, the most DEGs were observed in the ASM (470), naive (312), and TLM (333) B subpopulations, respectively (Figure 4—figure supplement 1A and Supplementary file 5). Specifically, among all the upregulated genes, the expressions of glycosylation-related, mitochondrial respiratory-related, and peptide chain elongation-related genes among the ASM, PB, and RM populations were drastically elevated after HTNV infection, including *RPN2*, *MT-ND6*, and *RPS26* (Figure 4—figure supplement 1B and Supplementary file 6). On the contrary, the upregulated genes in the naïve, DN, and IM populations were related to anti-inflammatory and immunosuppressive effects, inhibition of the antioxidative function, response to environmental stress, and B-cell growth and development, such as *TSC22D3*, *TXNIP*, *DUSP1*, and *KLF6*, whereas the downregulated genes played an important role in the inflammatory response, cell motility, and antigen presentation, including *LTB*, *ACTG1*, and *HLA-DRB5* (Figure 4—figure supplement 1C and Supplementary file 7).

Unexpectedly, we observed significant changes in the expression of glycosylation-related genes (GRGs) in the ASM, PB, and RM subclusters during HTNV infection compared to healthy controls. *PRN1* and *PRN2* were prominently upregulated, which encoded subunits of the oligosaccharyltransferase complex (Figure 4D). This implied that the process of glycosylation during HTNV infection was rapidly activated among the ASM and PB subclusters. In the ASM subcluster, the fold change

of GRGs from all four glycosylation modification-related genes, aside from *PRN1* and *PRN2*, was similar, suggesting that ASM may undergo complex and diverse glycosylation modification processes.

To enhance our understanding of the transcriptomic characteristics of GRG-high-expressing PB and ASM subclusters, we conducted a comprehensive pathway enrichment analysis and identified significant enrichment of pathways related to glycosylation modifications, including the endoplasmic reticulum protein-containing complex, rough endoplasmic reticulum, and protein N-linked glycosylation via asparagine (Figure 4E). Additionally, gene sets associated with glycosylation modifications showed positive regulation of these pathways (p=0.002, Figure 4F). These results indicated that glycosylation modifications in the ASM, PB, and RM subpopulations were dynamically regulated during the acute phase of HFRS, likely to satisfy the amplified anti-HTNV antibody requirement and accelerate viral clearance.

Discussion

IgG glycosylation is a key regulator of inflammatory responses for infectious diseases, acting as a molecular switch between anti-inflammatory and pro-inflammatory effects upon antigenic challenge. One of the most significant findings of this study is the elevation in IgG fucosylation and reduction in bisecting GlcNAc levels at the time of a 4-fold increase in the HTNV-NP-specific antibody titers. Afucosylation is known to enhance antibody-dependent cellular cytotoxicity (ADCC) by increasing the affinity of IgG-Fc for the FcγRIIIa receptor on natural killer cells, leading to heightened production of inflammatory cytokines such as IL-1β, IL-6, IFN-γ, and TNF-α (Larsen et al., 2021; Lu, Suscovich, Fortune, & Alter, 2018; Wieczorek, Braicu, Oliveira-Ferrer, Sehouli, & Blanchard, 2020). Increase in bisecting GlcNAc linked to Fc induces enhanced affinity for the FcγIIIa receptor and enhanced ADCC function of the

antibodies (Giron et al., 2020; Hou et al., 2021; Irvine & Alter, 2020). However, rather than directly influencing the Fc receptor binding, this modification enhances antibody functionality through afucosylation, which subsequently increases the binding affinity to the FcgRIIIa receptor (Ackerman et al., 2018; Jennewein & Alter, 2017). Our findings provide compelling evidence that the dynamic alterations in glycosylation patterns, characterized by increased fucosylation and decreased bisecting GlcNAc levels during viral infection, are critically involved in disease pathogenesis and clinical recovery, suggesting their dual utility as both diagnostic biomarkers and promising therapeutic targets.

We also observed elevated galactosylation levels in the acute phase compared to the convalescent phase, a phenomenon also reported in SARS-CoV-2 infections but not in chronic diseases and cancers (Hou et al., 2021; D. Liu et al., 2018). Increased galactosylation may expand the distance between CH2 domains, exposing key amino acids for FcγRIIIa binding and enhancing ADCC functions (Houde, Peng, Berkowitz, & Engen, 2010). Simultaneously, the increase in the ASM subset, which positively correlated with galactosylation, may reflect polyclonal activation of memory B cells driven by pattern recognition receptors and cytokines, leading to the production of galactosylated antibodies. Similarly, sialylation levels were higher in the acute patients, potentially linked to the expansion of the ASM and PB subsets. In this study, the PB subset was significantly elevated in the acute phase and positively correlated with sialylation, suggesting that rapidly proliferating plasmablasts may contribute to the secretion of sialylated antibodies. However, the mechanisms behind this require further investigation.

Our findings demonstrate that the IgG-Fc glycosylation plays a pivotal role in HTNV infection and rapid recovery. Fucosylated oligosaccharides and the absence of bisecting

GlcNAc are associated with favorable clinical outcomes. Importantly, specific B cell subsets, particularly ASMs and PBs, upregulated the secretion of galactosylated and sialylated antibodies, respectively. These findings would deepen our understanding of antibody glycosylation in anti-virus infection and provide a foundation for future studies aimed at optimizing glycoengineered therapeutic antibodies.

Materials and Methods

Study participants

Clinical specimens were collected from HFRS patients who were hospitalized in Baoji Central Hospital between October 2019 and January 2022. The HFRS patients were categorized into four clinical subtypes and clinical courses based on the previous reports (Ma et al., 2015; Tang et al., 2019; Zhang et al., 2022). This study was approved by the ethics committee of the Shandong First Medical University & Shandong Academy of Medical Sciences (R201937). Written informed consent was obtained from each participant or their guardians.

Single-Cell RNA Sequencing and public database

Flow cytometry antibody staining was conducted as previously described (Chakraborty et al., 2022). Dead cell and red blood cell were removed using the BD FACSAria™ III cell sorter, and PBMCs were collected for single-cell sequencing. The library construction was conducted as previously described (Jin et al., 2024).

We obtained single-cell transcriptome sequencing data from the Gene Expression Omnibus (GEO) database (GSE161354), containing six HFRS patients and two healthy volunteers, as well as healthy control samples from the Genome Sequence Archive (GSA) database (HRA000203), matched with patient demographic information.

Single-Cell Data Analysis

The FastQC software was used to evaluate the data obtained to ensure the quality of the

raw sequencing data. The raw data were mapped to the human reference genome (GRCh38, https://cf.10xgenomics.com/supp/cell-exp/refdata-gex-GRCh38-2020-A.tar.gz) using Cell Ranger. The Seurat R package (Version 4.4.0) was utilized for the merging and clustering of single-cell data. The Harmony R package and the anchor module of Seurat were used to remove batch effects between samples and groups for cell clustering. t-distributed Stochastic Neighbor Embedding (t-SNE) and Uniform Manifold Approximation and Projection (UMAP) were employed for dimensionality reduction and visualization of individual cells.

Cell Type Annotation

UMAP and t-SNE were used to reduce the dimension of all cells and to cluster them in a two-dimensional space based on shared features. The Single R (Version 2.2.0) package was used to independently infer the cell origin of each single cell based on a reference transcriptome dataset of pure cell types, for unbiased cell type identification from single-cell RNA sequencing data. Subsequently, specific highly expressed genes were combined with literature review for manual annotation to ultimately determine cell types. Classic biomarkers for specific cell types were used to identify cells in different clusters.

Differential Gene Identification and Functional Analysis

The *FindMarkers()* function in the Seurat package was used to identify differentially expressed genes (DEGs) between different cell groups, with criteria of $|\log2FC| > 0.25$ and p-value<0.05, pct.1>0.25. The ClusterProfiler R package was utilized for GO, KEGG, and GSEA enrichment analyses, and the ggplot2 and enrichplot R packages were used for visualization.

Measurement of HTNV specific antibodies

Levels of serum IgM and IgG antibodies to HTNV nucleocapsid protein (NP) were

assessed through the enzyme-linked immunosorbent assay (ELISA) kit (Wantai BioPharm, Beijing, China) in accordance with the manufacturer's instructions.

Analysis of IgG glycans

The process of isolating, labelling, purifying, and analyzing plasma IgG was performed using previously established methods (Hou et al., 2019; P. Liu et al., 2023).

Virus-specific B-cell detection

Virus-specific B-cell against HTNV were performed as previously described (Liechti & Roederer, 2019; Song et al., 2018).

Statistical analysis

Categorical variables were compared using chi-square tests. Normality of numeric variables was assessed with the Kolmogorov-Smirnov test; non-normally distributed continuous variables were analyzed using non-parametric tests. Statistical analyses were performed with R software version 4.1.1 (R Core Team, New Zealand) and GraphPad Prism 8 (GraphPad Software, San Diego, CA), with a significance level of $\alpha = 0.05$ (two-tailed).

Author contributions

Haifeng Hou and Weifeng Shi designed and supervised this study. Chuansong Quan, Lu Wang, Jiming Gao, Yaoni Li, Houqiang Li, Hongzhi Liu, Qian Li, and Liqiong Zhao collected the samples and performed experiments. Chuansong Quan, Lu Wang, Jiming Gao, Xiaoyu Xu, Zixuan Gao, Wenxu Ruan, and Weijia Xing analyzed the data and performed the statistical analyses. Lu Wang, Chuansong Quan, Jiming Gao, and Xiaoyu Xu wrote the manuscript. Haifeng Hou, Weifeng Shi, Weijia Xing, and Michael J. Carr reviewed and edited the manuscript. All authors had full access to all of the data in the study and accept responsibility for the decision to submit for publication.

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Data availability

The data underlying this article will be shared upon request to the corresponding author.

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database and Identifier
Chuansong Quan	2025	Glycosylated IgG antibodies accelerated the recovery of haemorrhagic fever with renal syndrome patients	https://www.ncbi.nlm.nih.gov/sra/PRJNA1238574	Sequence Read Archive, PRINA1238574

Zhang H, Wang Y (2022) **Increased CD4^{+}CD8^{+} double positive T cells during Hantaan virus infection** ID GSE161354. In the public domain at GEO http://www.ncbi.nlm.nih.gov/geo/. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE161354.

Zheng Y, Liu X (2020) **A human circulating immune cell landscape in aging and COVID-19** ID HRA000203. In the public domain at GSA https://ngdc.cncb.ac.cn/search/. https://ngdc.cncb.ac.cn/gsa-human/browse/HRA000203.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Figure legends

Figure 1. The flow chart of HFRS patient recruitment in Baoji Central Hospital in Shaanxi province from November 2019 to January 2022.

Figure 2. Immunophenotypic remodeling in the B-cell subsets during acute HFRS.

(A) t-distributed stochastic neighbor embedding (t-SNE) plot showing antibody secreting memory B cells (ASM), double negative (DN) B cells, intermediate memory cells (IM), marginal zone-like cells (MZB), naïve B cells, plasmablasts (PB), quiescent resting memory cells (RM), and exhausted tissue-like memory B cells (TLM) of PBMCs identified using an integrated and classification analysis. (B) t-SNE projection of canonical markers, including CD19, CD27, CD38, IgD, and IgM. (C) Proportions of the eight B cell subsets, colored by the healthy group (green) and HTNV groups (red). Boxplot features: minimum box, 25th percentile; center, median; maximum box, 75th percentile. (D) Frequency of the eight B cell subsets in between the healthy group (n = 8) and HTNV groups (n = 15). (E) FACS gating strategy for the measurement of the B-cell subsets: activated memory B cells ($CD21^-$ $CD27^+$, AM), RM B cells ($CD21^+$ $CD27^+$), IM B cells ($CD21^+$ $CD27^-$), TLM B cells ($CD21^-$ $CD27^-$), naive B cells ($CD27^-$ IgD^+), MZB B cells ($CD27^+$ IgD^+), ASM B cells ($CD27^+$ IgD^-), DN B cells ($CD27^-$ IgD^-), PB ($CD38^+$ $CD27^+$), and class-switched memory B cells ($CD38^-$ $CD27^+$, CSM), respectively. (F) The proportion of ASM, CSM, naive B, and PB cells in $CD19^+$ B cells in the acute and convalescent HFRS patients. Data are presented as mean ± SEM in panels C and F. *$p<0.05$ (Student's t-test).

Figure 3. Glycosylation modification of antibodies associated with HTNV infection. (A) Changes of different glycosylation types in both HTNV-NP specific IgG negative and positive plasma from 24 HFRS patients. (B) Differential IgG

glycosylation patterns across antibody titer levels quantified by ELISA. (C) Comparison of different glycosylation levels before and after the fourfold increase in the IgG antibody titers. *p<0.05, **p<0.01, ***p<0.001. For paired samples, Wilcoxon Signed Rank Test was used to assess the difference.

Figure 4. Glycosylation modifications of antibodies primarily deprived from antibody-secreting and plasmablast subpopulations.

(A) The correlation between the proportion of PB cells and the sialylation level. (B) The correlation between the proportion of ASM cells and the sialylation level. (C) The correlation between the proportion of ASM cells and the galactosylation level. (D) Dot plot shows the expression levels of glycosylation-related genes in the eight B cell groups. The pink color represents mannose related genes, the green color represents N-glycosylation related genes, the orange color represents galactosylation related genes, the purple represents sialylation related genes, and the red color represents fucosylation related genes. (E) Enriched pathways in the plasmablast and ASM subsets by GO enrichment analysis of the DEGs. (F) The GSEA map presents the enrichment score of GRGs in N-Glycan biosynthesis.

Supplementary materials

Supplementary file 1. The proportion of different B cell subpopulations between healthy controls and acute HFRS patients.

Supplementary file 2. Difference in the main IgG glycome features of HFRS patients between age groups.

Supplementary file 3. Difference in the main IgG glycome features of HFRS patients between sex groups.

Supplementary file 4. Differentially expressed genes of different B cell subgroups between healthy controls and acute HFRS patients.

Supplementary file 5. Differentially expressed genes of different B cell subgroups a between health and acute HFRS groups

Supplementary file 6. Differential expression genes in the ASM, PB and RM B cell subpopulations.

Supplementary file 7. Differential expression genes in the DN, IM and naive B cell subpopulations.

Figure 2—figure supplement 1. Single-cell transcriptomes of PBMCs from patients with HFRS.

(A) Uniform manifold approximation and projection (UMAP) presentation of the major peripheral immune cell types in the PBMCs from 15 HFRS patients and 8 healthy controls. (B) Dot plot shows the expression levels of canonical marker-related genes in the different subpopulations. (C) UMAP presentation of the major peripheral immune cell types among HFRS and healthy control groups. (D) Proportions of the eight cell subsets, colored by the healthy group (green) and HTNV groups (red). Boxplot features: minimum box, 25th percentile; center, median; maximum box, 75th percentile. (E)

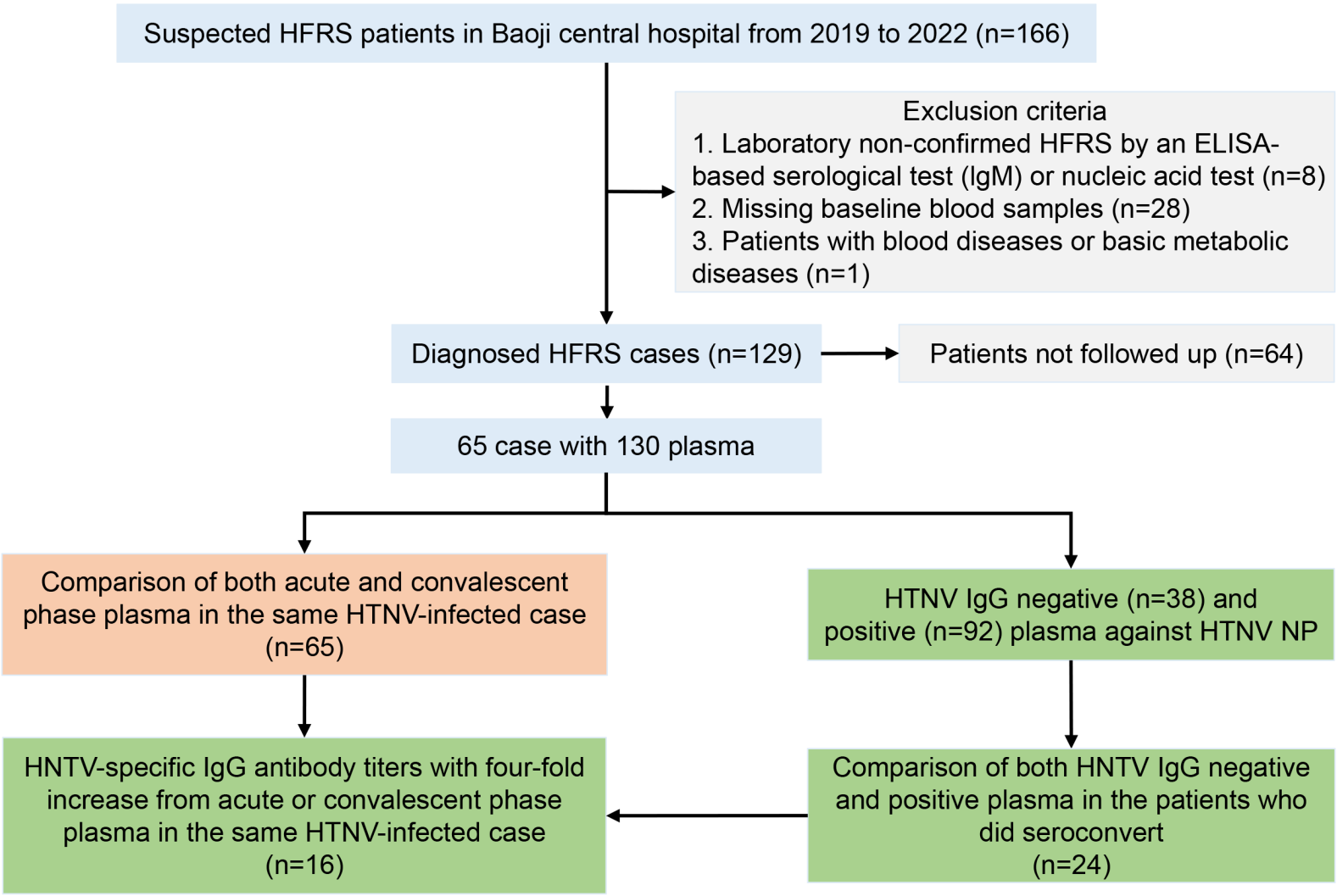
Frequency of the eight cell subsets in between the healthy group (n = 8) and the HTNV group (n = 15). (F) The UMAP plot shows the differentiation trajectories of different cell types calculated by pseudotime analysis.

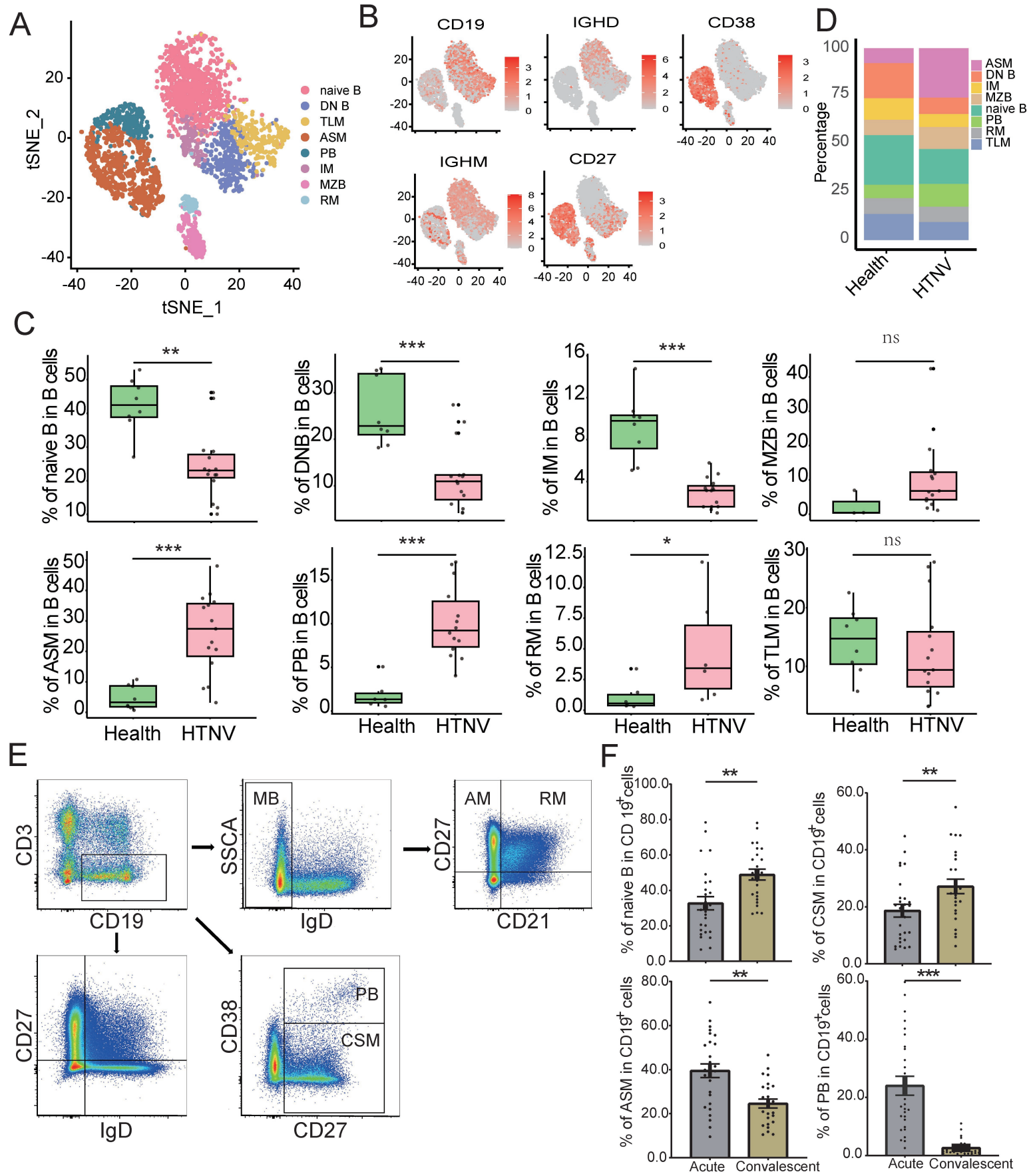
Figure 2—figure supplement 2. Dynamic analysis of the B-cell subsets in HFRS patients.

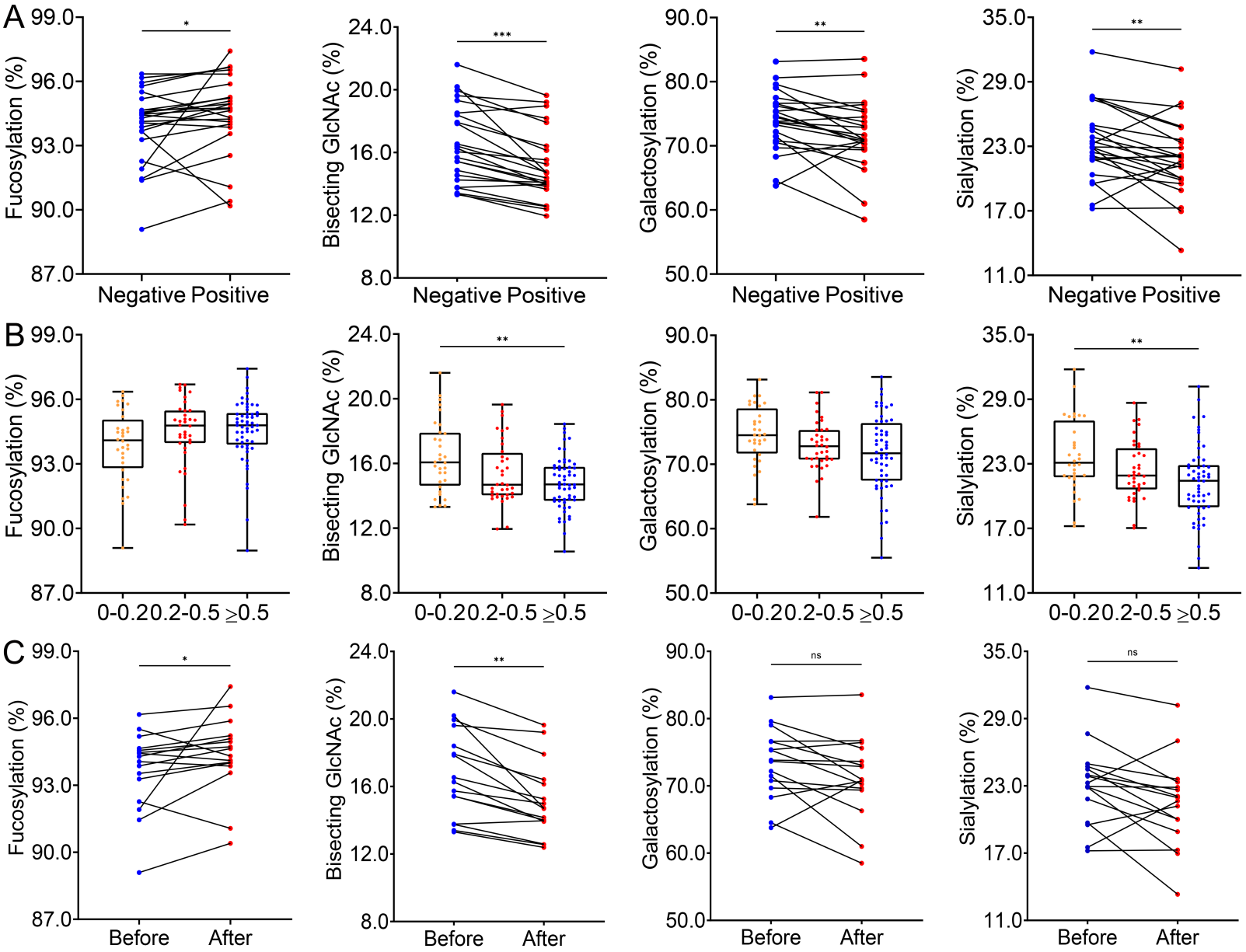
(A) Expression of MZB in $CD19^+$ B cells. (B) Expression of DN in $CD19^+$ B cells. (C) Expression of TLM in $CD19^+$ B cells. (D) Expression of AM in $CD19^+$ B cells. (E) Expression of RM in $CD19^+$ B cells. (F) Expression of IM in $CD19^+$ B cells.

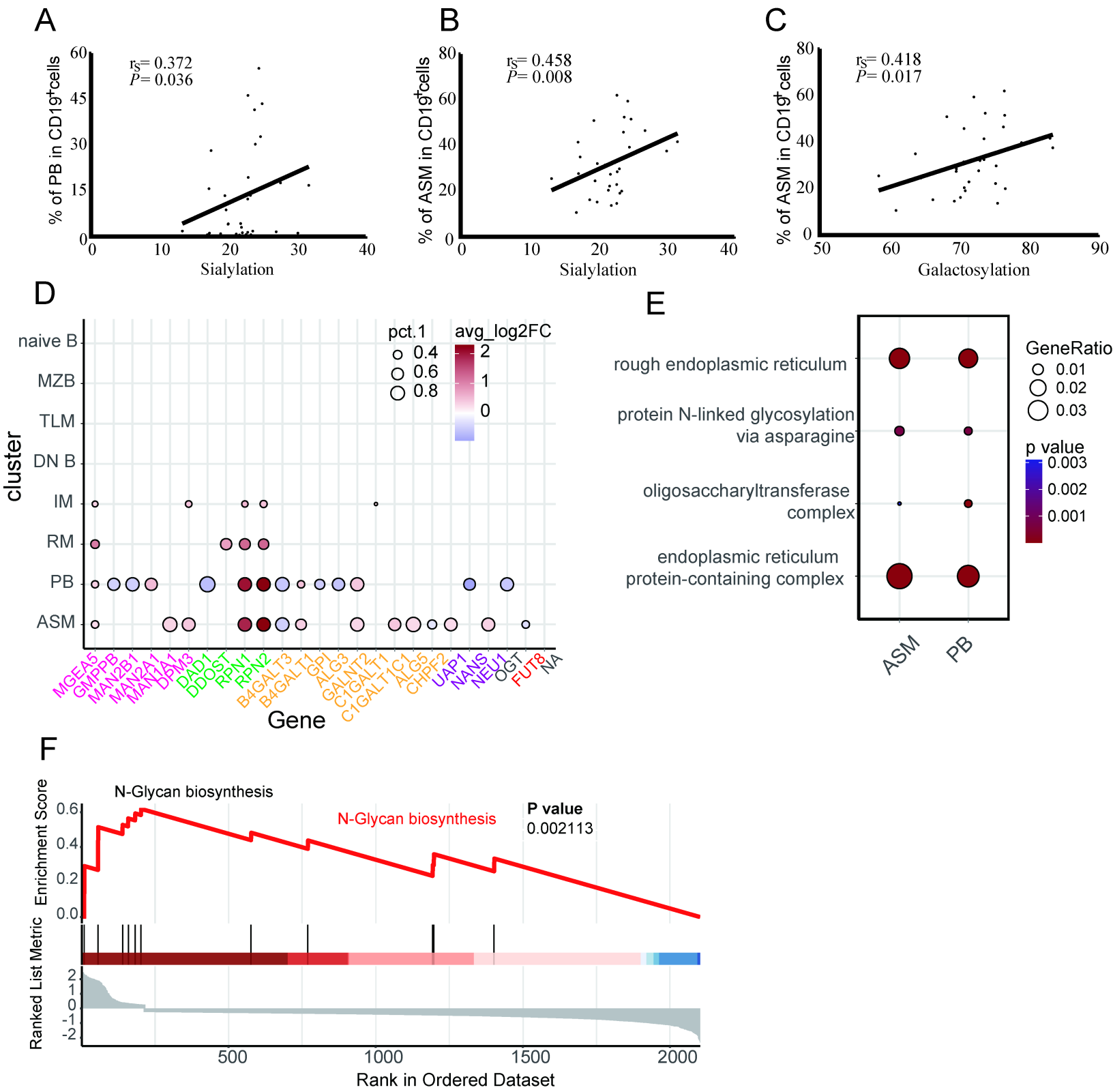
Figure 4—figure supplement 1. The differentially expressed genes and their functional changes in the B cell subsets post HTNV infection.

(A) The volcano plot shows the differentially expressed genes of different B cell subsets in acute HFRS patients. The red font represents the upregulation of the B cell subpopulations, and the blue font represents the downregulation of the B cell subpopulations. (B and C) The volcano plot displays the differentially expressed genes in upregulation B cell subpopulations including antibody secreting memory B cells, plasmablasts, and quiescent resting memory B cells (B), and downregulation B cell subpopulations including naive B cells, double negative B cells, and intermediate memory B cells (C).


Suspected HFRS patients in Baoji central hospital from 2019 to 2022 (n=166)
Exclusion criteria
1. Laboratory non-confirmed HFRS by an ELISA-based serological test (IgM) or nucleic acid test (n=8)
2. Missing baseline blood samples (n=28)
3. Patients with blood diseases or basic metabolic diseases (n=1)
Diagnosed HFRS cases (n=129)
Patients not followed up (n=64)
65 case with 130 plasma
Comparison of both acute and convalescent phase plasma in the same HTNV-infected case (n=65)
HTNV IgG negative (n=38) and positive (n=92) plasma against HTNV NP
HNTV-specific IgG antibody titers with four-fold increase from acute or convalescent phase plasma in the same HTNV-infected case (n=16)
Comparison of both HNTV IgG negative and positive plasma in the patients who did seroconvert (n=24)


A
tSNE_2
tSNE_1
naive B
DN B
TLM
ASM
PB
IM
MZB
RM
B
CD19
IGHD
CD38
IGHM
CD27
D
Percentage
ASM
DN B
IM
MZB
naive B
PB
RM
TLM
Health
HTNV
C
% of naive B in B cells
% of DNB in B cells
% of IM in B cells
% of MZB in B cells
% of ASM in B cells
% of PB in B cells
% of RM in B cells
% of TLM in B cells
Health
HTNV
ns
E
CD3
CD19
SSCA
IgD
MB
CD27
CD21
AM
RM
CD38
PB
CSM
F
% of naive B in CD 19+cells
% of CSM in CD19+cells
% of ASM in CD19+ cells
% of PB in CD19+cells
Acute
Convalescent


A
B
C
Fucosylation (%)
Bisecting GlcNAc (%)
Galactosylation (%)
Sialylation (%)
99.0
96.0
93.0
90.0
87.0
24.0
20.0
16.0
12.0
8.0
90.0
80.0
70.0
60.0
50.0
35.0
29.0
23.0
17.0
11.0
*
***
**
ns
Negative Positive
0-0.2
0.2-0.5
≥0.5
Before
After


A
% of PB in CD19+cells
rs= 0.372
P= 0.036
Sialylation
B
% of ASM in CD19+cells
rs= 0.458
P= 0.008
Sialylation
C
% of ASM in CD19+cells
rs= 0.418
P= 0.017
Galactosylation
D
cluster
naive B
MZB
TLM
DN B
IM
RM
PB
ASM
pct.1
0.4
0.6
0.8
avg_log2FC
2
1
0
MGEA5
GMPPB
MAN2B1
MAN2A1
MAN1A1
DPM3
DAD1
DDOST
RPN1
RPN2
B4GALT3
B4GALT1
GPI
ALG3
GALNT2
C1GALT1
C1GALT1C1
ALG5
CHPF2
UAP1
NANS
NEU1
OGT
FUT8
NA
Gene
E
rough endoplasmic reticulum
protein N-linked glycosylation via asparagine
oligosaccharyltransferase complex
endoplasmic reticulum protein-containing complex
ASM
PB
GeneRatio
0.01
0.02
0.03
p value
0.003
0.002
0.001
F
N-Glycan biosynthesis
Enrichment Score
N-Glycan biosynthesis
P value
0.002113
Ranked List Metric
Rank in Ordered Dataset