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Data-Driven Modeling for Precision Medicine in Pediatric Acute Liver Failure

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The absence of early outcome biomarkers for pediatric acute liver failure (PALF) hinders medical and liver transplant decisions. We sought to define dynamic interactions among circulating inflammatory mediators to gain insights into PALF outcome subgroups. Serum samples from 101 participants in the PALF study, collected over the first 7 d following enrollment, were assayed for 27 inflammatory mediators. Outcomes (spontaneous survivors (S, n = 61), nonsurvivors (NS, n = 12) and liver transplant patients (LTx, n = 28)) were assessed at 21 d post-enrollment. Dynamic interrelations among mediators were defined using data-driven algorithms. Dynamic Bayesian network inference identified a common network motif, with HMGB1 as a central node in all patient subgroups. The networks in S and LTx were similar, and differed from NS. Dynamic network analysis suggested similar dynamic connectivity in S and LTx, but a more highly interconnected network in NS that increased with time. A dynamic robustness index calculated to quantify how inflammatory network connectivity changes as a function of correlation stringency differentiated all three patient subgroups. Our results suggest that increasing inflammatory network connectivity is associated with nonsurvival in PALF and could ultimately lead to better patient outcome stratification.

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INTRODUCTION

Pediatric acute liver failure (PALF) is a complex, dynamic and rapidly evolving clinical syndrome whose onset is not completely understood (1,2). Identifying patients likely to recover spontaneously or die without liver transplant (LTx), or not benefit from LTx, is necessary to inform LTx decisions. Unfortunately, clinical or laboratory biomarkers that predict outcome reliably are not available. As there is evidence of immune or inflammatory dysregulation in acute liver failure (3–7) associated with outcome, we hypothesize that markers of immune or inflammatory dysregulation in PALF could be leveraged to predict outcome.

Computational modeling of complex biological systems is emerging as a tool to investigate the unpredictable behavior of biologic pathways (8), including those pathways operant in inflammation (9,10). Inflammation, similar to many complex biological phenomena, is manifest by networks of interacting mediators that can be inferred to be interactive via various computational algorithms (11,12). This concept is especially important given the volume of data needed to define drivers and biomarkers of complex diseases (10,13). Recently, we utilized computational modeling techniques with measured circulating inflammatory mediators to describe dynamic mediator networks that suggest novel interactions and possible biomarkers in the setting of trauma and sepsis (14–17). Using similar techniques, we have also demonstrated that PALF participants who survive with their native liver exhibit inflammatory network structures similar to LTx recipients (18).

Opportunities associated with computational modeling of inflammatory responses in PALF fall into two broad categories. First, patterns of inflammation

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and biological interactions may be associated with clinical outcomes. Current models to predict death or spontaneous survival in PALF using clinical, biochemical and demographic data are inadequate (19,20), and it is unlikely that a single inflammatory mediator determined at a single time point along the dynamic PALF trajectory would be sufficient to reliably predict outcome (6). Therefore, computational modeling analyses could fill a critical void in medical decisions. Second, identifying potential regulatory points in dynamic networks of immune or inflammatory dysregulation could identify opportunities for selective therapeutic interventions.

To address these two opportunities, we hypothesize that key mediators involved in inflammatory interactions, as well as subgroup-specific dynamic patterns of these mediators, can distinguish relevant patient outcomes. Identifying these dynamic interconnections of selected immune and inflammatory cytokines could serve to distinguish among PALF outcome groups, enhance LTx decision-making, identify disease mechanisms and suggest novel therapeutic opportunities for PALF. Herein, we sought to extend our previous studies in PALF (18) to determine if a broader and deeper computational analysis, based on a larger dataset, would yield further insights.

MATERIALS AND METHODS

Selection of PALF Patients

This study was approved and carried out in accordance with the guidelines of the Institutional Review Boards at each participating institution listed in Acknowledgments, and the National Institutes of Health provided a Certificate of Confidentiality. Participants were enrolled in the PALF Study if they met the entry criteria (1) (see Supplementary Materials and Methods) and after written informed consent was obtained from the children's parents or guardians. After enrollment, demographic and clinical data were recorded daily for up to 7 d, with a single daily serum sample for research scheduled to be collected on the calendar day of enrollment (d 0) or with the first morning blood draw following enrollment and daily for up to 7 d (d 1–d 7), or until death, LTx or discharge from hospital. Outcomes were assigned on d 21 as survival without LTx (S), death without LTx (NS) or LTx. Since not all patients in each outcome group had research samples obtained on the same days, patients were selected for this analysis if they had at least three daily samples with at least 100 μL of serum available.

Assays of Inflammatory Mediators

We measured 25 cytokines and chemokines that serve as biomarkers for the complex inflammatory response using the Luminex™ 100 IS system (Luminex) and the Human 25-plex® Luminex beadset (Millipore). This antibody bead kit includes eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α2, IFN-γ, interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, soluble IL-2 receptor α chain (sIL-2Rα), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IFN-γ-inducible protein of 10 kDa (IP-10; CXCL10), monocyte chemotactic protein-1 (MCP-1; CCL2), monokine induced by γ-interferon (MIG; CXCL9), macrophage inflammatory protein (MIP)-1α, MIP-1β, and tumor necrosis factor (TNF)-α. In addition, we assayed HMGB1 using a commercially available enzyme-linked immunoabsorbent assay (ELISA) (Shino-Test) and the nitric oxide (NO) reaction products $NO_2^- + NO_3^-$ using the nitrate reductase method (Cayman Chemical).

Statistical Analysis and Data-driven Modeling

Our analytic strategy was to apply a stepwise series of statistical and data-driven modeling techniques aimed at discovering principal drivers, interconnected networks and potential key regulatory nodes based on data on systemic inflammation. For the analyses, we used the raw data (time series for each individual) without applying any transformation. We detail these analyses below.

Dynamic Bayesian network (DBN) inference was carried out to define the most likely single network structure that best characterizes the dynamic interactions among systemic inflammatory mediators across all time points, in the process suggesting likely feedback structures that define central nodes. The networks might also suggest possible mechanisms by which progression of the inflammatory response differs within a given patient subgroup. In this analysis, time courses of unprocessed inflammatory mediator measurements from each patient were used as input for a DBN inference algorithm, implemented in Matlab® essentially as described previously for gene array data (21) and modified by our group for the study of systemic acute inflammation (18,22,23).

Dynamic network analysis (DyNA) was carried out to define, in a granular fashion, the central inflammatory network nodes as a function of both time and patient subgroup. The main difference between DyNA and DBN is that DyNA allows for granular temporal resolution of networks over distinct time intervals, while DBN helps suggest feedback structures. Using inflammatory mediator measurements of at least three time points for each patient, networks were created over seven consecutive time periods (d 0–1, d 1–2, d 2–3, d 3–4, d 4–5, d 5–6, d 6–7) using Matlab® software (15,18). Connections, defined as the numbers of trajectories of serum inflammatory mediators that move in parallel, were created if the Pearson correlation coefficient between any two nodes (inflammatory mediators) at the same time interval was greater or equal to a threshold ranging from 0.7 to 0.95, as indicated. The network complexity for each time interval was calculated using the following formula: sum $(N_1 + N_2 + \ldots + N_n)/(n-1)$, where N represents the number of connections for each mediator and n is the

total number of mediators analyzed. The total number of connections represents the sum of the number of connections across all time intervals for all patients in a given subgroup.

Dynamic robustness index was calculated to quantify how network connectivity (as defined by DyNA) changes as a function of correlation stringency in each patient subgroup over seven consecutive time periods. This metric was calculated for each time period and patient subgroup using the lowest (0.70) and highest (0.95) correlation stringency and network connectivity (C) as follows: $(C_{0.7}\text{-}C_{0.95})/(0.95–0.7)$.

All supplementary materials are available online at www.molmed.org.

RESULTS

Clinical Criteria and Demographics of PALF Participants

There were 1,144 patients enrolled in the PALF study at the time of this analysis. We restricted our cohort to those with at least three daily samples containing at least 100 μL of serum and identified 101 participants: S (n = 61), NS (n = 12) and LTx (n = 28). The demographic data for study participants are shown in Supplementary Table S1. Nonsurvivors were younger than survivors and LTx recipients. The schematic of the statistical and computational methods and the rationale for their use in this study are depicted in Supplementary Figure S1.

Differential Trajectories of Inflammatory Mediators in Different PALF Outcomes

Time courses of 27 mediators (shown in Supplementary Figure S2) identified median levels of eotaxin, IL-6, IL-8, IL-10, IL-15, IP-10, MCP-1, MIG and NO (measured as NO_2^-/NO_3^-) with distributions that significantly differed ($P < 0.05$) among the three PALF subgroups (Supplementary Table S2).

DBN Analysis Identifies a Common Signature in PALF

With a much larger cohort than our previous report (18), we utilized DBN inference to determine if mediator feedback structures and possible regulatory architectures in inflammatory networks of S, NS and LTx recipients could be discerned from the time courses of circulating inflammatory mediators in PALF participants. A key feature of dynamic networks is the presence of mediator feedback, which can be inferred algorithmically, so we focused on mediators that exhibit self-feedback as central nodes, similar to our recent studies in PALF, trauma and sepsis (15,17,18,24). Results of this analysis on each of the three PALF subgroups are shown in Figure 1. Though data were segregated by outcome group before being subjected to DBN inference, the algorithm did not make assumptions about the connectivity of the network in any outcome group. In NS, DBN inference suggested a primary network driven by a core motif of HMGB1, which was inferred to affect the downstream production of MCP-1, IL-8, IP-10 and MIG. In contrast, in both the S and LTx groups, the DBN pattern suggested a network regulated by switching between HMGB1 and the chemokines IP-10 and MIG, each of which drives its own expression. In the S and LTx groups, HMGB1 was inferred to affect the downstream production of sIL-2Rα and MCP-1. Collectively, and in agreement with our prior study (18), we found similar inflammatory networks in the S and LTx groups that differ from the NS, which suggests the presence of a common inflammatory signature associated with clinical outcomes in liver inflammatory diseases such as PALF.

Dynamic Network Analysis of Circulating Inflammatory Mediators Serves to Differentiate PALF Patients

We next sought to define, in a more granular fashion, the time evolution of dynamic networks of systemic inflammation of PALF participants. Accordingly, we employed DyNA (14,15) to discern and compare the interconnections among inflammatory mediators in the PALF subcohorts over defined ranges of time. We hypothesized that patients with worse clinical outcomes would have more robust dynamic networks of inflammation, in line with the concept of pathology driven by self-sustaining inflammation. To quantify the robustness of a given dynamic network, we performed DyNA using increasing stringency levels from 0.7 (a correlation value commonly used to characterize trajectories that move in parallel either up or down) (14,15) up to 0.95, as shown in Supplementary Figure S3. This analysis resulted in a marked decrease in the number of significant mediators and connections in all groups, though with greater complexity in NS. Subsequently, we repeated the detailed DyNA using a stringency level of 0.95 (Figure 2). DyNA showed an overall low and similar network pattern and total number of connections over all time intervals in the S and LTx groups. However, a much more complex network pattern (Figure 2A) and higher total number of connections (Figure 2B) were observed in NS. Furthermore, the dynamic connectivity of systemic inflammation in NS rose constantly over time, while remaining essentially unchanged in S and LTx patients (Figure 2C).

Figures 3A–C show individual mediators and their connectivity in the three patient groups using a stringency level of 0.95. This new analysis served to identify the mediator(s) and mediator networks characteristic of the PALF subcohorts and, more importantly, to clearly distinguish the LTx from the S group based on relatively subtle differences (Figures 3B–C). This analysis, reflected also in Figure 3C, reinforced the hypothesis of self-sustaining or self-propagating inflammation in NS versus a lower-level, chronic or self-resolving inflammatory response in S and LTx. In addition, this analysis suggested subtle differences in the inflammatory mediators involved in S versus LTx responses.

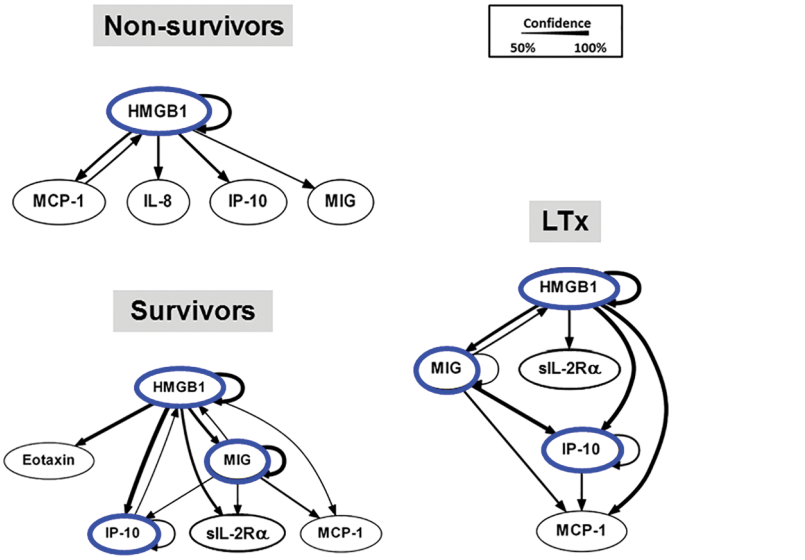

Figure 1. Dynamic Bayesian Network (DBN) analysis of circulating inflammatory mediators in PALF patients. Circulating inflammatory mediators in serum samples from PALF spontaneous survivors (S, n = 61 patients), nonsurvivors (NS, n = 12 patients) and liver transplant recipients (LTx, n = 28 patients) were measured and DBN analysis was performed, as described in Materials and Methods. Inflammatory mediators are shown as nodes, and the arrows connecting them suggest an influence of one mediator on the one(s) it is connected to. The arrows do not distinguish positive from negative influences. Semicircular arrows suggest either positive or negative feedback of a given mediator on itself.

We next hypothesized that network robustness was higher in NS versus S and LTx, and that this feature underlies the time-dependent rise in inflammatory connectivity in NS. As shown in Figure 4, this hypothesis was supported by analysis of a dynamic robustness index, calculated to quantify how inflammatory network connectivity changes as a function of correlation stringency, which showed that NS had higher network robustness at all time intervals compared with S and LTx. The robustness index was lowest in LTx, intermediate in S and highest in NS.

Because nonsurvivors, as a group, were significantly younger than survivors (see Supplementary Table S1), we considered age as a possible confounder when analyzing mediator time courses. To rule out the role of age, first we selected all survivors (n = 45 patients) whose ages, as a group, were not statistically significantly different (P = 0.053) from those of the nonsurvivors as shown by the median and Q1–Q3 [S: 3.3, 1.1–9.9]. Similar to the whole S cohort (n = 61 patients), in the age-matched survivors DyNA demonstrated a significant decrease in the total number of connections and network complexity compared with NS (Figure 5). Similarly, as for the whole S cohort, the DBN pattern of this smaller group suggested a network regulated by switching between HMGB1 and the chemokine MIG, each of which drives its own expression (Supplementary Figure S4).

DISCUSSION

Our core finding is that nonsurvival in PALF is associated with rising complexity of dynamic inflammation networks, and that this rising complexity appears to be due to higher network robustness in NS patients. The study of robustness of biological networks is of great interest from an evolutionary and systems engineering perspective (25). In the present study, we used a robustness index derived from DyNA to quantify how inflammatory network connectivity changes as a function of correlation stringency in the dynamic networks observed in PALF patients, reasoning that a higher degree of inflammatory network robustness reflects a greater resistance to self-resolution, and likely to therapy as well. While DyNA further reinforced the similarity between S and LTx suggested by DBN inference and demonstrated a very low degree of network connectivity across time at a high stringency of correlation, the robustness index clearly differentiated among the three PALF subgroups. Our findings suggest either that inflammation observed in S and LTx is at a chronic and relatively stable level, or that small flares of inflammation are resolved. In contrast, NS exhibited a strikingly different pattern: network connectivity was already higher than S and LTx upon initiation of the study and rose steadily through d 7. Examination of the connected mediators in NS suggests a major inflection point of systemic inflammation between d 2 and d 3 after enrollment, leading to a large increase in connectivity out to d 7. Chemokines and DAMPs such as HMGB1 appear to play an early role in nonsurvivors, with later inflammation involving Th1, Th2 and Th17 pathways. These findings suggest that a large number of inflammatory pathways and mechanisms are operant in patients destined not to survive PALF, leading to a state of self-sustaining or self-propagating inflammation driven by both innate and T cell–dependent mechanisms.

Our computational analysis using established dynamic network discovery algorithms (DBN, DyNA) also repeatedly points to a number of mediators (e.g., HMGB1, MIG, IP-10, IL-6 and MCP-1) as possible biomarkers in PALF. These analyses suggest that dynamic patterns of systemic inflammation could reflect networks, which in turn identify key inflammatory mechanisms operant in, or characteristic of, PALF. Our present findings support and extend prior findings regarding dynamic networks of systemic inflammation in PALF, namely that S and LTx exhibit similar dynamic networks that differ from those of NS PALF patients (18).

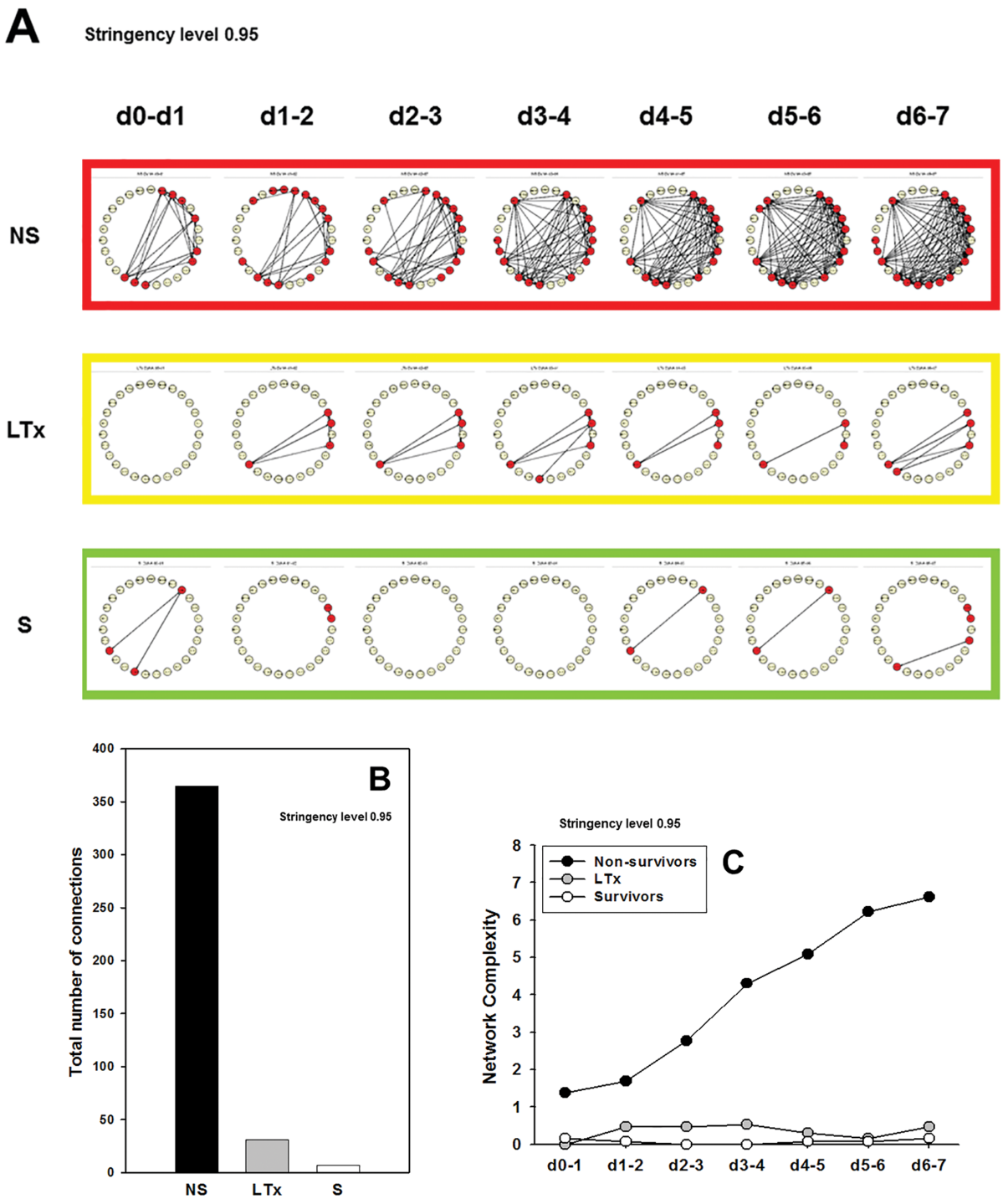

Figure 2. Dynamic network analysis (DyNA) of circulating inflammatory mediators in PALF patients. Circulating inflammatory mediators in serum samples from PALF spontaneous survivors (S, n = 61 patients), nonsurvivors (NS, n = 12 patients) and liver transplant recipients (LTx, n = 28 patients) were measured and DyNA (stringency level = 0.95) was performed during seven time frames, d 0–1, d 1–2, d 2–3, d 3–4, d 4–5, d 5–6, d 6–7, as described in Materials and Methods. Panel A shows an overview of all the networks and mediator connections (closed circles represent mediators with at least one connection to another mediator; open circles represent mediators that had no connections to other mediators as determined by DyNA). Panel B represents the total number of connections for each group of patients over all time intervals. Panel C shows the network complexity for each group of patients during each of the seven time frames calculated, as described in Materials and Methods.

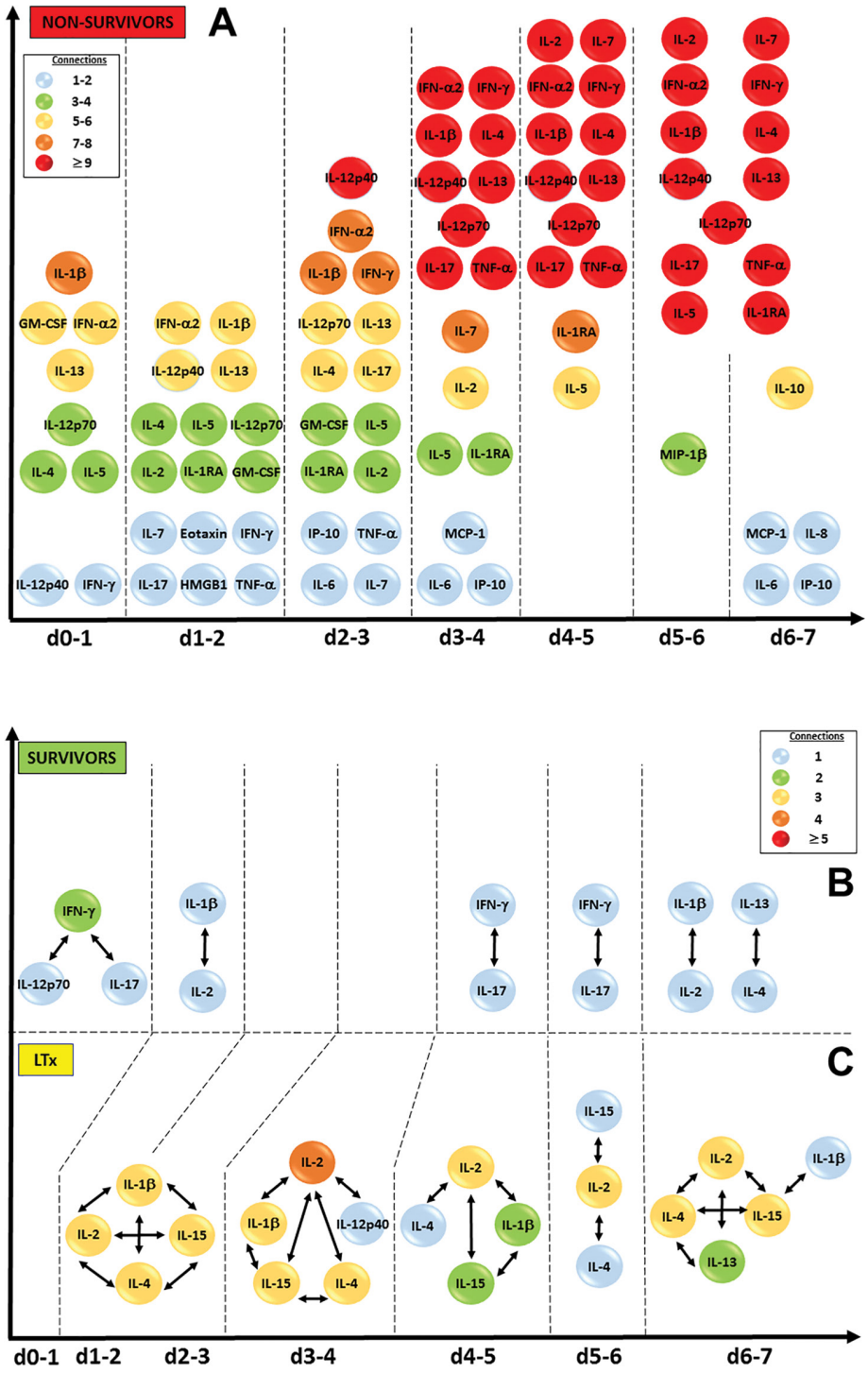

Figure 3. Significant circulating inflammatory mediators and connections in PALF patients (DyNA, high stringency). Circulating inflammatory mediators in serum samples from PALF patients were measured and DyNA (stringency level = 0.95) was performed during seven time frames, d 0–1, d 1–2, d 2–3, d 3–4, d 4–5, d 5–6, d 6–7, as described in Materials and Methods. Results show the significant mediators based on dynamic patterns and network connectivity in three main groups of PALF patients (panel A: nonsurvivors, n = 12; panel B: LTx recipients, n = 28; and panel C: spontaneous survivors, n = 61).

Several mediators emerged as possible drivers or biomarkers of interest in PALF based on our combined analyses: HMGB1, MIG, IP-10, IL-6 and MCP-1. HMGB1 not only plays a critical intracellular role as a DNA chaperone, chromosome guardian, autophagy sustainer and protector from apoptotic cell death, but also senses and coordinates the cellular stress response as the prototypic alarm/danger molecule (26). Our network analyses suggest an inflammatory chain reaction that includes HMGB1, which exhibits self-feedback behavior that may act as a central node in all PALF subgroups. In this dynamic network, HMGB1 is inferred to interact with the chemokines MIG, IP-10 and MCP-1. Elevated MIG and IP-10 mRNA has been reported in liver failure (27,28), and circulating levels of both chemokines have been implicated in chronic hepatitis C (29). We have shown that MCP-1 is a central node in hepatocyte inflammatory responses to stress, a role perhaps related to the ability of circulating MCP-1 levels to segregate outcomes in human blunt trauma (15). Interestingly, in addition to our prior demonstration of the central role of MCP-1 in stressed hepatocytes (15), greatly increased MCP-1 levels were observed in livers from patients with fulminant hepatic failure (30). We demonstrated that IL-6 was elevated but not connected to other inflammatory mediators in dynamic networks in mice undergoing trauma/hemorrhage (14). In this study, IL-6 was also elevated with very low connectivity in NS, but not in S and LTx, suggesting that inflammatory hypoconnectivity, like hyperconnectivity, might be a hallmark of pathology.

Increased HMGB1 levels have been reported in patients with ALF, suggesting that HMGB1 is released from injured liver tissue (31). Although a specific role for HMGB1 in PALF has not previously been reported, the observed HMGB1-centered network in all PALF groups, and finding HMGB1 to be present in early inflammation networks prior to a massive increase in network connectivity in NS, might suggest a common DAMP/chemokine inflammatory

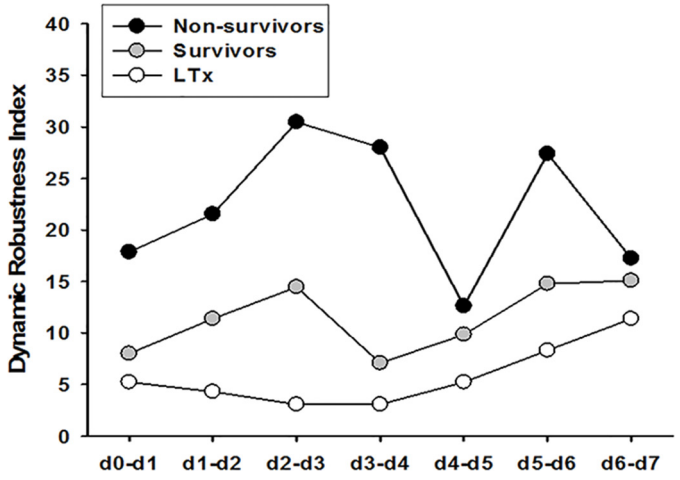

Figure 4. Dynamic robustness index in PALF. Circulating inflammatory mediators in serum samples from PALF patients were measured. DyNA (stringency levels = 0.7 and 0.95) was performed during seven time frames, d 0–1, d 1–2, d 2–3, d 3–4, d 4–5, d 5–6, d 6–7, and the dynamic robustness index for each patient group (survivors (n = 61 patients), nonsurvivors (n = 12 patients), LTx recipients (n = 28 patients)) was calculated, as described in Materials and Methods.

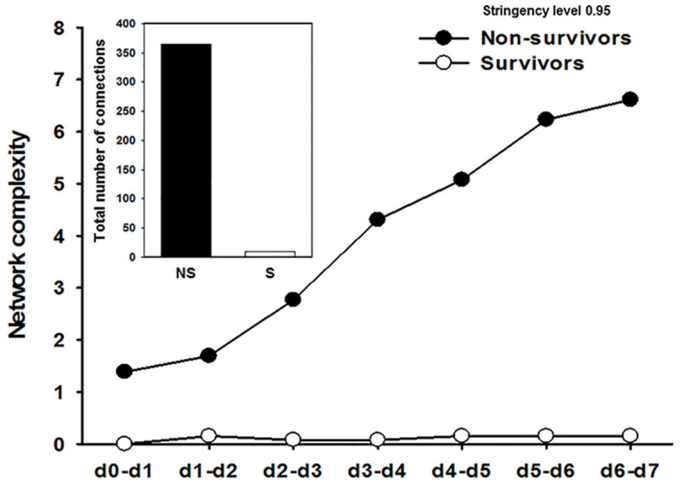

Figure 5. Dynamic network analysis (DyNA) of circulating inflammatory mediators in PALF patients (age-matched survivors versus nonsurvivors). Circulating inflammatory mediators in serum samples from PALF spontaneous survivors (S, n = 45 patients) that closely matched nonsurvivors (NS, n = 12 patients) were measured and DyNA (stringency level = 0.95) was performed, as described in Materials and Methods. The figure shows the network complexity during each of the seven time frames calculated, as described in Materials and Methods, and the total number of connections for each group of patients over all time intervals (inset).

process driving liver injury in PALF. Thus, HMGB1 might represent a novel therapeutic target in PALF.

There are some unavoidable limitations to our study. First, unlike other clinical settings, such as traumatic injury, we were unable to determine the onset of systemic inflammation in PALF patients, as the onset of PALF cannot be determined. With only 8 d of samples analyzed, changes in dynamic networks before or after this time period still need to be identified. The same applies to samples from participants with short, mild events or severe, rapid progression to death or transplantation who were excluded from this analysis, given the requirement for at least three available samples. In addition, our analysis was not able to determine if individuals can transition between a "survival" network to a "nonsurvival" network. Unsupervised hierarchical clustering based on unprocessed inflammatory mediator data (not shown) could not segregate patient subgroups, raising a cautionary note that standard statistical analysis of mediators singly or as a group might be of limited use for predicting patient outcomes. Finally, although we identified a set of mediators in the context of dynamic networks that could potentially be considered as outcome biomarkers, we acknowledge the difficulties involved in translating these complex analyses and hypotheses into a diagnostic test.

The present results validate the core findings from our previous study of PALF (18) and also suggest the presence of overly robust self-sustaining and highly connected inflammation networks in nonsurvivors. Despite a heterogeneous inflammatory response in PALF participants, these studies suggest that a network-based analysis has the potential to clearly segregate spontaneous survivors and LTx recipients from nonsurvivors, and spontaneous survivors from LTx recipients. While similarities between S and LTx groups also suggest that some of the LTx patients might have a "survivable" disease, differences with the NS group points to a distinct group of patients who might not benefit from LTx. Our study also suggests a common inflammatory network regulated by HMGB1 and the chemokines IP-10 and MIG, which might represent either a general "liver signature" in inflammatory conditions such as PALF or the central contribution of the liver to other inflammatory diseases.

CONCLUSION

Identifying immune/inflammatory networks that reflect dynamic changes in the inflammatory response could lead to opportunities for diagnosis or directed therapeutic intervention, enhance liver transplant decisions and improve patient outcomes in PALF. In this regard, our studies also identified biomarkers such as HMGB1 as potential therapeutic targets in PALF. More generally, the use of novel computational methodology could streamline studies of other inflammatory diseases.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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