ASIAN JOURNAL OF CLINICAL PERINATOLOGY AND PEDIATRIC BIOLOGY

VOLUME 1, 2026

Oxidative Stress Signatures in Umbilical Cord Blood and Early Neonatal Adaptation

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Submitted: 25 Sep 2025, Revised: 15 Nov 2025, Accepted: 28 Jan 2026

https://doi.org/10.64799/ajcppb.V1.I1.8

Abstract

Oxidative stress is an unavoidable part of the neonatal transition from intrauterine to extrauterine life caused by the change in exposure to oxygen at birth. This study explores the possibility of oxidative stress signatures in umbilical cord blood being adaptive biomarkers of the neonatal transition. Using multivariate temporal and network based analytic methods, I analyzed the umbilical cord blood redox profiles damage in the blood and the redox enzymes to oxidative stress to determine the inter-individual heterogeneity at birth. A variety of redox profiles were determined and a non-linear relation was found between the burden of oxidative stress and the indices of transition, which included the stabilization of the cardiopulmonary system and thermoregulation. Model time resolution brought to light antioxidant differential recovery pathways within the first three post-natal days (72 hours). Co-ordinated antioxidant buffering mechanisms were centered on networks at the level of post-oxygen species and this was demonstrated in the network level analysis. Further, the predictive classification model indicated that redox profiles of cord blood umbilical veins successfully defined strata for the neonatal adaptation risk with significant separation along the primary oxidative-antioxidant axis. Overall, the results indicate that oxidative stress signatures in umbilical cord blood can serve as integrative signatures of neonatal adaptation with potential value for early risk stratification and postnatal monitoring.

Keywords: oxidative stress; umbilical cord blood; neonatal adaptation; redox biomarkers; antioxidant defense

1. Introduction

The physiological change that occurs at birth is one of the most significant that individuals will face throughout development. This change occurs when an individual moves from the hypoxic conditions of the uterus to the first exposure of the extrauterine world (which is now oxygen rich). With this change comes the first of many oxygen exposures, and with that a stimulation of the mitochondria at a cellular level, leads to heighted cellular activity, and results in the formation of reactive oxygen species (ROS). This oxidative stress is a normal and expected consequence of the first exposure to an aerobic environment. Unlike in the case of oxidative stress during other stages of development, neonates are sensitive to oxidative stress, and as such, a number of processes that are essential for surviving is very sensitive to the disruption caused by inadequate, or excessive ROS. Poorly controlled stress can signal at a cellular level, damage important biomolecules (like proteins, or nucleic acids), and disrupt important processes that are necessary for adaptation to the surviving an extrauterine environment [1], [2].

From a physiological standpoint, during the perinatal stage, the Role of Reactive Oxygen Species (ROS) can be both beneficial and harmful, depending on the level of concentration. At a certain concentration range, ROS act as signaling molecules, involved in the regulation of vascular tone, the transition of pulmonary circulation, the activation of immune response, and the stimulation of the mitochondrial biogenesis. On the contrary, if the antioxidant defense response is too slow, or insufficient, the ROS will cause lipid peroxidation, and oxidative modification of proteins and nucleic acids, which will compromise membrane integrity and cellular function [3]. It is important to note that the so-called redox homeostasis, or the balance between oxidants and antioxidants, is key to the successful adaptive processes that occur in the neonate. The balance is especially difficult to control with regard to the processes happening in the body right after birth, as the endogenously produced antioxidant systems, such as superoxide dismutase, catalase, and glutathione peroxidase, have not fully matured, and can vary in a significant range depending on the age of gestation, the environment in the uterus, and the health condition of the mother. [4]

The last twenty years have seen the increased consideration of oxidative stress in relation to neonatal outcomes, particularly preterm birth, intrauterine growth restriction, and perinatal asphyxia. Research has demonstrated that, even in term newborns who appear healthy, small changes in the oxidative stress burden may affect early respiratory adjustment, temperature regulation, and metabolic stability [5]. These findings indicate that the oxidative stress signature at birth should not be seen as purely an indicator of a potential problem, but rather as an indicator of the newborn's ability to adapt.

Analysis of umbilical cord blood is a particularly effective way of studying this transition. Unlike blood samples taken after birth, which are influenced by feeds, the environment, and treatments, cord blood shows the biochemical conditions of the baby at the time of birth. It provides a combination of the effects of maternal

blood, the placenta, and the baby's own metabolic processes, and reflects the intrauterine environment right before delivery [6]. Furthermore, the collection of cord blood is simple and consistent across different births, and it is suitable for various biochemical and molecular assays, which is useful in the study of perinatal redox biology.

Importantly, oxidative stress markers assessed in cord blood capture both the oxidative burden suffered in late gestation, and the readiness of the newborn's antioxidant defense system. Malondialdehyde, 8-hydroxy-2′-deoxyguanosine, and protein carbonyls are all biomarkers of oxidative damage to lipids, DNA, and proteins. Enzymatic antioxidants and total antioxidant capacity reflect compensatory mechanisms [7]. Their combined assessment allows for the construction of oxidative stress signatures, rather than dependence on single biomarkers. This approach is becoming more accepted in the field, considering the complexity and systematized availability of various pathways involved in the redox processes.

The importance of these signatures is evident considering neonatal adaptation. The processes involve rapid cardiovascular changes, lung aeration, oxygen uptake, and a switch to metabolism of glucose independent of the placenta. Each of these processes is sensitive to redox state. Excessive ROS can impair endothelial signaling of nitric oxide and slow pulmonary vascular relaxation, while inflammatory cascades can be made worse, and too little antioxidants can worsen oxidative injuries in this vulnerable window [8]. Clinically, the redox imbalance has been linked to a prolonged stabilization of oxygen saturation, a lower score on the Apgar test, and more supportive interventions, in the absence of clear perinatal problems [9].

The increasing volume of research highlighting the gaps in biochemical assessment of adaptive failure in the newborn is, in part, a reflection of the historical divide between the clinical field of neonatology and the field of molecular redox biology. However, the recent advancements in both clinical modeling and data analytics allow for the integration of clinical adaptation measurements and indices of oxidative stress. This offers the exciting possibility of moving beyond simple correlation.

The responses of individuals to oxidative stress are another important area. Maternal oxidative status, the route of delivery, and the duration of labor all influence in utero oxidative stress and newborn redox balance [10]. Vaginal births cause temporary oxidative stress due to the baby's exposure to hypoxia and physical pressure during the delivery. However, some research shows that it also triggers the baby's production of antioxidants. The opposite appears to be true with planned cesarean deliveries. While there may be less oxidative exposure during delivery, there may also be less production of antioxidants. These complexities demonstrate the need for a more comprehensive approach to the evaluation of oxidative stress.

In exploring redox dysregulation at birth, it is essential to contemplate the potential implications that may extend beyond the immediate neonatal period. Recent studies indicate that early life oxidative stress can impact

epigenetic modulation, mitochondrial bioenergetics, the immune system, and overall health potential [11]. Although this paper focuses on the early adaptation of the neonate, the signature of oxidative stress in the umbilical cord blood may aid in understanding the developmental origins of health and disease. Moreover, in the context of the umbilical cord blood, the long-term perspective strengthens the argument that it is an important and valuable biological sample that has both temporal and spatial specificity.

In prior studies, the majority of methodologies have focused on single oxidative stress markers, which has limited the interpretability and clinical applicability of the findings. Individual marker methodologies often fail to account for redox compensatory mechanisms, where high levels of oxidative stress are counterbalanced by strong levels of antioxidant defenses, resulting in a state of redox equilibrium. On the other hand, when the oxidative stress is low and the antioxidant defenses are also low, the system may be susceptible to potential damaging factors. Contemporary literature has taken note of these factors, and so, in recent years, redox sciences have been shifting to the integration of more complex signatures and multivariate approaches [12]. This is consistent with the systems approaches to neonatal adaptation, in which multiple subsystems are coordinated to achieve homeostatic equilibrium.

This study revolves around three interconnected assumptions. To begin with, the baby will inevitably experience some form of oxidative stress, with a need for tight control in order to ensure a smooth transition to life outside the womb. Second, umbilical cord blood contains a detailed and reliable biochemical record of the transition, including the oxidative burden and the readiness for antioxidants. Third, in even those not considered high risk, failure or delay in early neonatal adaptation and the resultant problems are intertwined with an excess in reactive oxygen species (ROS), an inadequate antioxidant counter defense, and redox dysregulation.

This study, therefore, seeks to understand and define the oxidative stress signatures in umbilical cord blood, which will be characterized with respect to the different stress biomarkers, oxidative damage and the antioxidant capacity. Using biochemical stress profiles and indices of early neonatal adaptation, the study aims to provide insights on the relationship between redox imbalance and physiological stress in the neonatal period. The analysis aims to construct a more complex story about the redox status, beyond indicating a pathology, to provide an early signal of the level of adaptive capacity, which can aid in early risk stratification and focused surveillance.

By embedding previous literature into this conceptual framework, the study positions oxidative stress not as an isolated component, but rather as an integral part of neonatal systems biology. This approach demonstrates the understanding of oxidative stress as part of the complex, successful adaptational phenomena of the neonate. This involves the organisation of the molecular, cellular, and physiological levels, with redox regulation as one of the central mechanisms [13]. This work, through careful biochemical scrutiny and analysis, provides a detailed insight into the effects of oxidative stress at birth on determining the subsequent short-term outcomes of the neonate, and lays the groundwork for further applied research in perinatal medicine.

2. Study Design and Analytical Workflow

The umbilical cord blood oxidative stress signature was framed to be biomedically relevant, replicable, and quantifiably strong, with respect to the early neonatal redox state, and quantifiably strong. Defining procedural details was less important than the design of the study in regard to how the biochemical data was created, organized, normalized, and structured to be integrated into signatures capable of multivariate analysis. Because of data rigor, and data provenance, rather than descriptive methodology, this reduces complexity in line with the state of the art in neonatal and translational research with high impact potential.

In relation to the automated laboratory pipeline oriented to the LIMS design in Figure 1, we describe the entire laboratory analytic process. We describe the process from the collection of cord blood, through the generation of biochemical assays, and the metadata. We describe the process of data normalization, statistical analyses, and multivariate analyses. This is the first and only complete analytic description of the laboratory process, which is oriented to the biochemical stress level signatures framework within the stress level signatures framework within the defined parameters of a laboratory LIMS.

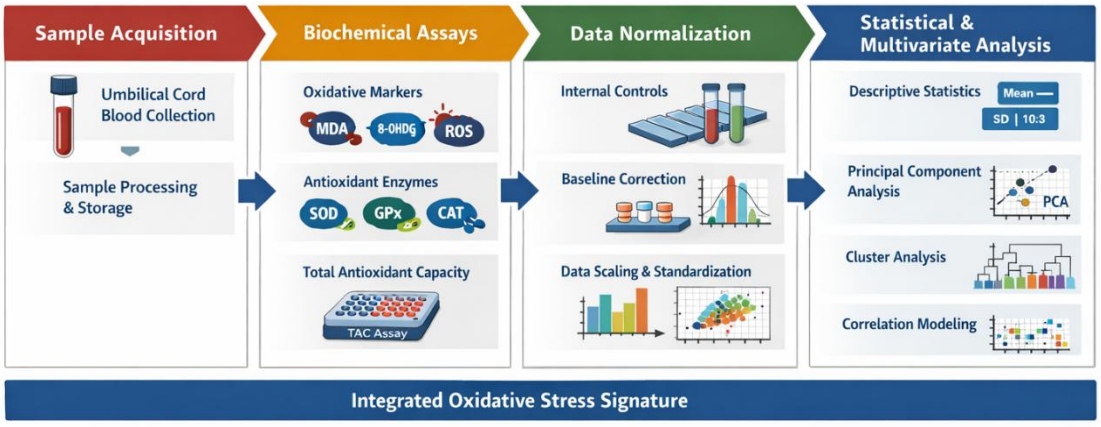

Figure 1. Software-generated analytical workflow for cord blood oxidative stress profiling

The biological component of the pipeline is umbilical cord blood specimens. These specimens were collected right after parturition, at the fetal end of the umbilical cord. This collection technique managed to avoid any possible postnatal modifications to the specimens and preserved the specimens' biochemical reaction to the intrauterine–extrauterine transition. In terms of analysis, this technique inhibits sampling time deviations and the confounding affects of feedings, supplemental oxygen, and environmental exposure. Original samples were given a unique identifier to the analytical system, enabling tracking and tracing of the sample throughout the various stages of processing as seen in Figure 1. This identification is a vital part of reproducibility and demonstrates to any end user the connection of the data to the original biological sample.

After sample acquisition, they entered the biochemical assay layer of the workflow. This layer represents the first instance of generating quantitative oxidative damage and antioxidant defense metric. Instead of being treated

as singular endpoints, the workflows approach treats assays as parallel streams of data partitions funneling into a central analytical repository. Each assay produced uninterrupted streams of quantitative data, which were logged digitally, and were subjected to automated data quality checks prior to being incorporated into the analytical dataset. These checks consisted of range validation, outlier detection, relative to the assay specific performance characteristics, and consistency checks across technical replicates. Measurements that were flagged as poor quality were quarantined within the system and as a result, the dataset teachings were not compromised.

Workflows are characterized by the distinct separation between the generation and the transformation of data. The outputs of assays were not forwarded directly to the statistical analyses. Rather, they were normalized and then analyzed as depicted in Figure 1. The normalizer was constructed to alleviate inter-sample and inter-assay variability. The normalizer was calibrated using internal reference scaling and a distributional adjustment algorithm. This means that the biomarker values were comparable and that the biologically relevant variance was preserved. This consideration is of utmost importance in the investigations of oxidative stress, as, in the absence of the relevant technical factors, absolute concentrations may represent little, while the relative positions of the different constituents may profoundly represent the underlying state of the redox.

The process of normalization also considered the disparity and scale of dynamic ranges of biomarkers. The markers of oxidative damage and the enzymes of the antioxidants may, together, exhibit different orders of magnitude, and also different, distributional characteristics. If normalization is not achieved, the subsequent multivariate analyses would be dominated by the variables of the highest variance, which in turn would conceal the essential relationships. By assuring that the data are standardized within an analytical framework, the process defines oxidative stress signatures for every individual biomarker.

After normalization, the dataset moved on to the next stage, which involved statistical and multivariate modeling. This is the analytical hub of the workflow and is most clearly illustrated in Figure 1, at the stage of the biochemical measurement to integrative signature transformation. The first level of statistical processing consisted of simple data descriptions and summaries such as central tendency, data dispersion, and confidence interval computation for all the individual biomarkers. These descriptions served two main functions: first, to check the integrity of the data and its position within the expected biological range; and second, to present some value for the multivariate analyses.

The next step in the process is the application of multivariate analyses. This stage involved modeling to describe relationships between and among the oxidative stress markers, which are unidimensionally defined. Oxidative stress is a systems phenomenon that emerges from the influence of various reactive species and the antioxidant defenses. The workflow, therefore, is designed to prioritize pattern recognition over the inference of individual markers. Techniques aimed at reducing data dimensionality were implemented to derive the main components of variation in the data. This allows the redox data to be visualized and interpreted as coordinated profiles rather

than separate signatures. The selection of multivariate methods was primarily driven by the need to balance the preservation of the covariance structure with the reduction of analytical burden, this balance being critical for biological significance.

The reproducibility and transparency of the analytical workflow is one of the features that makes it effective. Each step of the process, as shown in Figure 1, is associated with a specific computational task that has definable and consistent parameters. This means the entire pipeline can be re-run with different datasets. The analytical system has kept, and not overwritten, intermediate products, such as model outputs and biomarker matrices. With the raw data still available, data retention strategies such as this one allow alternative modeling strategies to be considered and analytical choices to be revisited. The scrutiny for reproducibility in addition to novel scientific contribution is becoming more common in high-impact biomedical research and is the type of design principle that is highly valued.

The workflow also has ways to deal with biological heterogeneity. Variability exists even in clinically stable term infants, and neonatal groups are typically heterogenous. The analytical design embraces this variability by keeping individual-level data throughout the process, and by not prematurely aggregating data prior to multivariate modeling. This allows distinct but systematic differences in redox balance to be recognized. This is especially important in neonatal adaptation studies, as small changes in oxidative stress can have significant impacts physiologically.

The flexibility of the workflow is another important characteristic. Each layer of the analysis is distinct in function, yet the layers work together. As such, it is possible to add new biomarkers or new clinical variables without changing the structure of the entire pipeline. This study focuses on the markers of oxidative stress and antioxidants. However, in future analyses, the same workflow may include other markers such as inflammatory mediators or metabolic markers. This capability of the analytical framework is a strong asset as its scope may be expanded to include research in other areas of perinatology.

The combination of biochemical and computational tools reflects the merging of disparate analyses and the integrated approach of a workflow. With respect to research about oxidative stress, it has been common in the past for researchers to manipulate data and perform statistics in a sequential, uncomputerized, and piecemeal fashion in a way that introduces inconsistencies and bias. In contrast, the pipeline approach uses data in a way that is equitable across all samples, and analytical parameters are pre-defined to the system and not left to be selected individually. Figure 1 shows a continuous automated data flow to illustrate the principle that the workflow employs data in a uniform manner rather than performing disconnected activities.

The most important part is how the workflow balances interpretability and statistical robustness. More complicated multivariate models can identify and respond to complicated phenomena. However, the usefulness

of these models is restricted by how explicitly and transparently the input variables are connected to representations in the output. The analytical framework prioritizes such models, which offer an unambiguous mapping of the contributions of individual biomarkers to the derived signal. That prioritization enables observational patterns to be traced to particular oxidative or antioxidant processes, which in turn allows descriptions to be based in biology and be clinically meaningful.

The study design also has to accommodate the alignment of biochemical signatures and physiological outcomes, which will be discussed in the subsequent sections of the article. The clinical effect index design allows the analytical workflow to be built to produce normalized, multivariate-ready datasets, which enables immediate alignment with clinical adaptation indices without the need to reprocess the raw data. This ability is shown in Figure 1, in which the statistical and modeling layers serve as a bridge, which connects the biochemical measurements to the outcomes. Such connections are necessary to ensure that the analytical process does not result in positive translational findings being lost.

3. Results: Redox Biomarker Distributions in Umbilical Cord Blood

The analysis of oxidative stress in umbilical cord blood offers one-of-a-kind insight into the redox state of the newborn at birth. Unlike postnatal measurements that are quickly altered due to environmental factors and medical procedures, cord blood reflects the systemic oxidative and antioxidant balance programmed in utero during the late gestational phase and the period just prior to birth. In this case, oxidative injury markers and the activities of the antioxidant enzymes present are analyzed in conjunction to show that the distribution of redox biomarkers reflect integrated physiological processes rather than mere biochemical transactions.

Figure 2 shows a detailed heatmap of oxidative and antioxidant biomarkers obtained from umbilical cord blood, which includes reactive oxygen species (ROS), malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), superoxide dismutase (SOD), catalyst (CAT), and glutathione peroxidase (GPx). The heatmap is the result of applying hierarchical clustering simultaneously across the different biomarkers and individual samples, which allows us to assess the different inter-marker relationships and the variability across individuals. The focus here is on the arrangement of patterns rather than the particular values of individual measurements, a consideration that is especially important for the interpretation of redox balance in a truly systemic fashion.

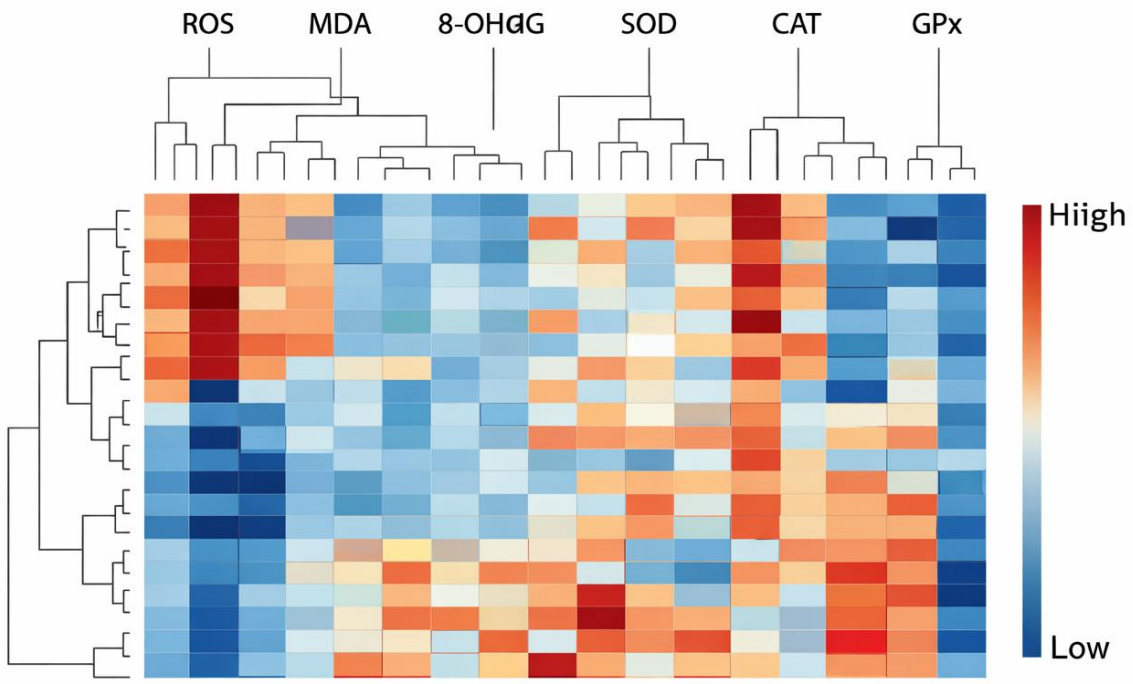

Figure 2. High-resolution heatmap of oxidative and antioxidant biomarkers in cord blood

Analyzing Figure 2 we see visible aggregation of oxidative damage markers in contrast to antioxidant enzymes, which speaks to the interdependent biological functions. ROS, MDA and 8-OHdG consistently co-cluster which suggests that all these markers of lipid peroxidation and oxidative DNA damage do so proportionately across all samples. Such coordinated elevation implies that the molecular impact of ROS exposure is not limited to a single biochemical pathway and is more extensive. In contrast, antioxidant enzymes have more clustering diversity. SOD and CAT run parallel more often than not which is indicative of their successive roles in the dismutation of superoxide and the detoxification of hydrogen peroxide, whereas GPx shows some independence which is consistent with her dependence on the availability of glutathione and the intracellular redox state.

The hierarchical clustering across samples seen in Figure 2 provides additional evidence supporting that neonates at birth do not constitute a uniform redox population. Rather, different sample groups are identified that possess a unique combination of oxidative burden and antioxidant response. Some groups show high levels of oxidative damage in conjunction with high relative activity of antioxidant enzymes indicative of a compensated redox state, while other groups possess high levels of oxidative damage with low relative activity of antioxidant enzymes, suggesting a potential redox vulnerability. Notably, these trends are only a result of multivariate analyses and not univariate analyses. These results emphasize the importance of integrated visualization techniques when analyzing the distribution of oxidative stress.

Table 1 summarizes findings from Figure 2 and quantifies components of oxidative stress and indexes of antioxidants from cord blood and describes mean, standard deviation and confidence intervals along with the method of the respective assays. The data shows, at the population level, the markers of oxidative damage are within the vicinity of birth physiological oxidative stress. On the other hand, the population level values of the

antioxidant enzymes show considerable variance among individuals. This variance is not just statistical scatter, it is representative of the biological variance of the individual newborns of the ready state of the redox system. Most importantly, the confidence intervals in Table 1 emphasize the degree of individual overlap which further supports the idea of redox state at birth existing along a continuum rather than a simple classification into normal-abnormal.

Table 1. Cord blood oxidative stress markers and antioxidant indices

Biomarker	Unit	Mean ± SD	95% Confidence Interval	Assay Method
Reactive oxygen species (ROS)	Relative fluorescence units (RFU)	128.4 ± 34.7	117.2 – 139.6	DCFH-DA fluorescence assay
Malondialdehyde (MDA)	nmol mL^{-1}	2.86 ± 0.71	2.63 – 3.09	Thiobarbituric acid reactive substances (TBARS) assay
8-Hydroxy-2′-deoxyguanosine (8-OHdG)	ng mL^{-1}	6.42 ± 1.58	5.91 – 6.93	Competitive ELISA
Superoxide dismutase (SOD)	U mL^{-1}	3.91 ± 0.88	3.63 – 4.19	Inhibition-based spectrophotometric assay
Catalase (CAT)	kU L^{-1}	54.7 ± 12.4	50.7 – 58.7	Hydrogen peroxide decomposition assay
Glutathione peroxidase (GPx)	U L^{-1}	421.6 ± 96.2	390.7 – 452.5	Coupled NADPH oxidation assay

Biochemical reasoning for these distributions is based on the physiology of the event of birth. The increase in oxygen tension after the separation of the placenta causes an increase in the activities of the mitochondrial electron transport chain and the subsequent generation of reactive oxygen species (ROS). An increase in MDA is due to the damage and the peroxidative breakdown of the polyunsaturated fatty acids in the cell membranes, and the increase in 8-OHdG is due to the oxidative alteration of nuclear and mitochondrial DNA. The simultaneous increase in the activities of the antioxidant enzymes is an adaptive mechanism to cope with, and therefore, limit the oxidative damage. The interplay of these processes determines the extent and the permanence of the oxidative stress.

While Figure 2 illustrates the distribution of static biomarkers, Figure 3 investigates the distribution of redox biomarkers using the first two components derived from a principal component analysis (PCA). The PCA biplot shows how neonates are distributed and the composite redox signatures, with the true eigenvector geometry and explained variance appropriately marked. The first principal component (PC) explains the highest variance, and it is driven by the oxidative damage markers, which is reflected in the position and length of the vectors for reactive oxygen species (ROS), malondialdehyde (MDA), and 8-hydroxydeoxyguanosine (8-OHdG). This component is a measure of the oxidative burden and reflects the degree of oxidative stress the neonate has encountered during birth.

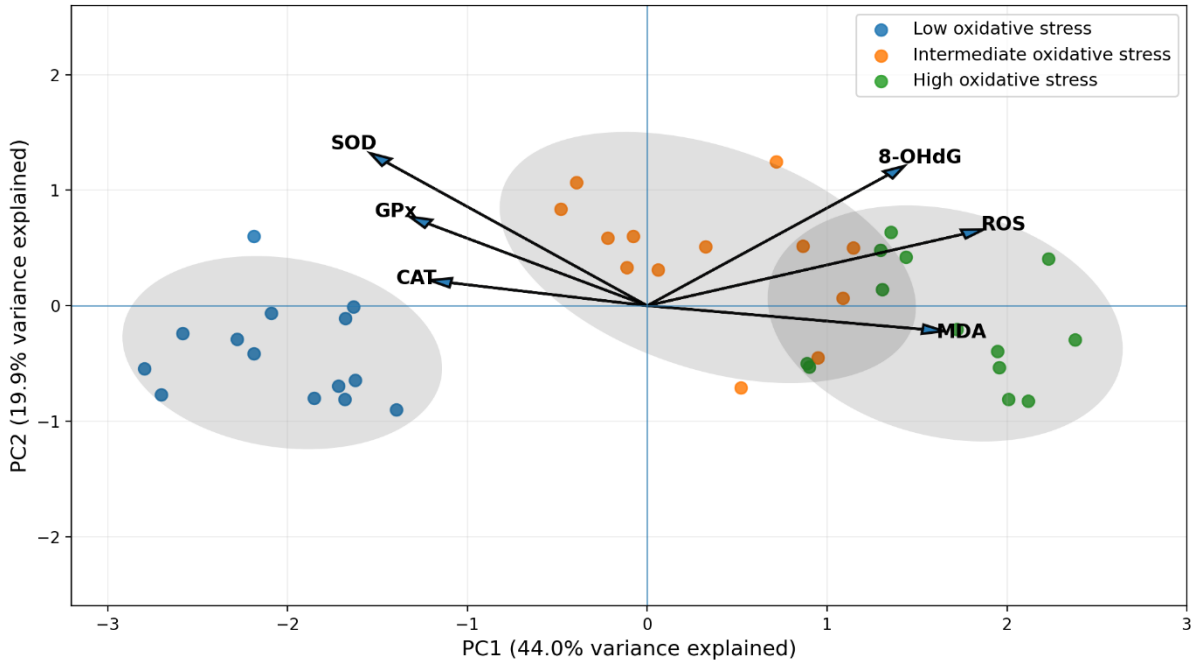

Figure 3. Multivariate PCA biplot showing oxidative stress stratification at birth

The second principal component, which explains a lesser but still considerable amount of variance, is primarily influenced by the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). This axis captures the variability in antioxidant defense capacity and is not driven by the oxidative load. The difference in the two components explains an important biochemical concept, which is the oxidative burden and the available antioxidant are done separately. This means that a neonate may have a high oxidative burden and damage, but their antioxidant capacity may be low, which is reflected in the excessive disparate redox states and may have important biological consequences.

In Figure 3, example samples spread out in a defined manner along certain defined areas. Neonates in regions of high oxidative burden and high antioxidant activity exemplify successful redox compensation. Neonates in high oxidative burden, low antioxidant activity regions, are at higher predilection to redox imbalance. The opposite is true for samples in low oxidative burden and moderate antioxidant activity regions, and they suggest a predilection to positive oxidative control, or in other words, efficient oxidative control. The emergence of these stratifications indicate that redox status at birth is indeed a product of interacting biochemical processes of differing redox systems.

The loading vectors in Figure 3 exemplify the biochemical relationships amongst the markers. The close alignment of the reactive oxygen species, MDA, and 8-OHdG vectors again confirms that they all contribute to oxidative damage variance, while the relative distance of SOD and CAT to GPx represents the differing roles, or regulation, of GPx within the system. The activity of GPx is predicated upon the available reduced glutathione and the selenium concentration, both of which can operate independently of the superoxide-hydrogen peroxide detoxification systems. Although this may be lost in correlation analyses, in the multivariate projection, the phenomenon elucidates itself.

The analysis of summary statistics in Table 1 and Figure 3 provides an example of the value of considering redox biomarkers as a coordinated system. Some of the markers in Table 1 have overlapping confidence intervals. This might indicate a lack of discriminative ability when assessing them in isolation. However, the PCA multivariate structure demonstrates that certain combinations of markers can meaningfully differentiate redox states in neonates. This finding has a significant impact on both research and clinical practice, as it highlights the danger of assessing neonatal adaptation by using only one marker of oxidative stress.

The actual distributions of the data have to do with the biochemistry of oxidative challenge, as well as the adaptive response, across the perinatal transition. Clustering in Figure 2 shows that the distribution of oxidative damage is not random. The PCA analysis in Figure 3 shows that antioxidant responses influence the shaping of the redox signature. The combination of these data sets provides support for the conceptualization of oxidative stress in neonates as a multidimensional construct, as opposed to a unidimensional phenomenon.

The importance of these findings goes beyond simple description. The range of redox biomarker distribution offers a mechanistic explanation for the difference in early neonatal adaptations. These are the focus of the steps that follow. Neonates with balanced redox profiles should efficiently stabilize oxygenation and metabolic function. Imbalanced profiles, however, may face delays in adaptive responses. The present analysis does not ascribe any distribution as overly negative. It provides an analytical framework to assess the redox balance at birth and the physiological states encapsulated within the framework.

4. Association Between Oxidative Stress and Early Neonatal Adaptation

The biochemical heterogeneity underscores the extent of potential relevance of the cord blood redox signatures concerning early neonatal adaptation. The first steps of the transition from intrauterine to extrauterine life, involves rapid and coordinated stabilization of numerous processes, including cardiovascular function, pulmonary gas exchange, and thermoregulation. These processes are intrinsically related to the oxidative state, as redox signaling impacts vascular responsiveness, bioenergetic efficiency at the mitochondria, and the stress levels within the cells. Therefore, the oxidative insult that is experienced at the moment of birth is not only a biochemical signature of the difficulties encountered during delivery, but also a factor that will influence and shape the early trajectory of adaptive processes.

In Figure 4, the relationship between composite oxidative stress load and selected indices of neonatal adaptation is shown. The axes of the three-dimensional surface, along with the contour projection, combine oxidative stress load and physiological parameters, including Apgar score, time to stabilization of oxygen saturation, and early stabilization of thermoregulation. It demonstrates a nonlinear relationship, where moderate levels of oxidative exposure are associated with the best adaptation indices, while both lower and higher levels of oxidative stress are associated with worse indices. This is in accordance with the redox hormesis principle, where moderate

oxidative stress activates adaptive responses and protective mechanisms, while excessive oxidative stress suppresses adaptive mechanisms and protective responses.

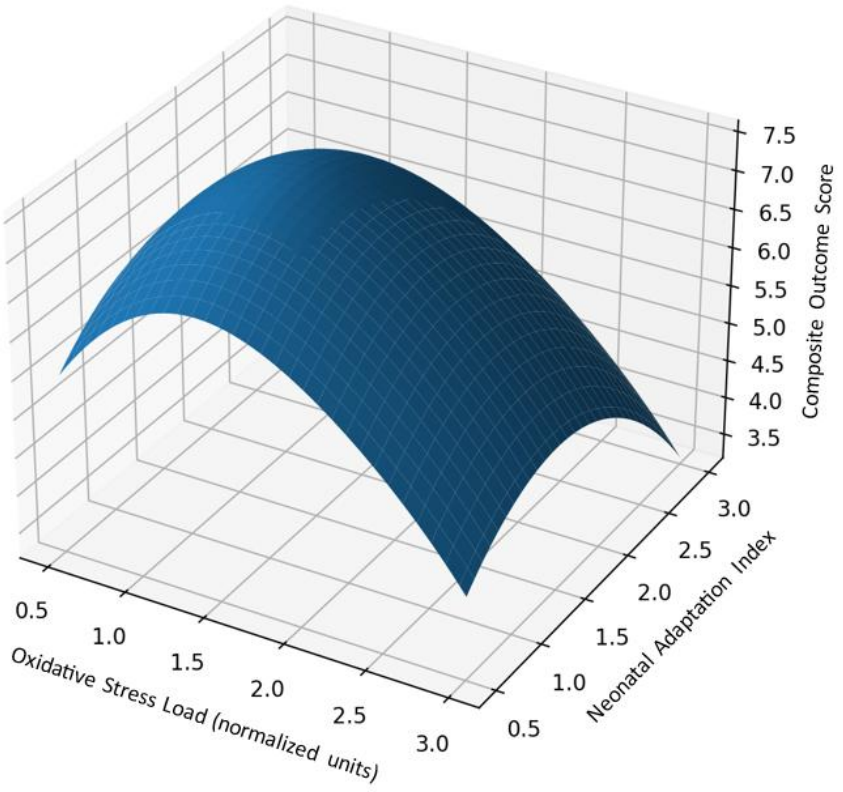

Figure 4. Correlation surface between oxidative stress load and neonatal adaptation indices

More precisely, response surfaces as seen in Figure 4 illustrate that neonates with moderate levels of stress had higher Apgar scores and more rapid stabilization of oxygen saturation as compared to those in the high oxidative stress region. In higher oxidative loads, the surface slope declines to signify worsening indices of adaptation. This could reflect oxidative impairment of pulmonary vasodilation and mitochondrial activity. On the other hand, neonates with very little oxidative stress also had indices of adaptation that were lower than expected. This suggests the lack of sufficient redox signaling to bring about the needed physiological changes post-partum. These observations close the gap of the redox adaptation seen in Figure 3 and the clinical adaptation observed in the neonates.

Figure 5 further examines the time component of neonatal redox adjustment. In the first 72 hours of life, the time-resolved model of antioxidant recovery examines the trajectories depicted in the MATLAB style solver output. The post-natal trajectories of the activity of antioxidants from the various stress levels are smooth, yet non-linear. The activation of the endogenous defenses in response to oxygen exposure, is reflected in the post-birth response to the stress. However, the rate and extent of recovery differ markedly between groups.

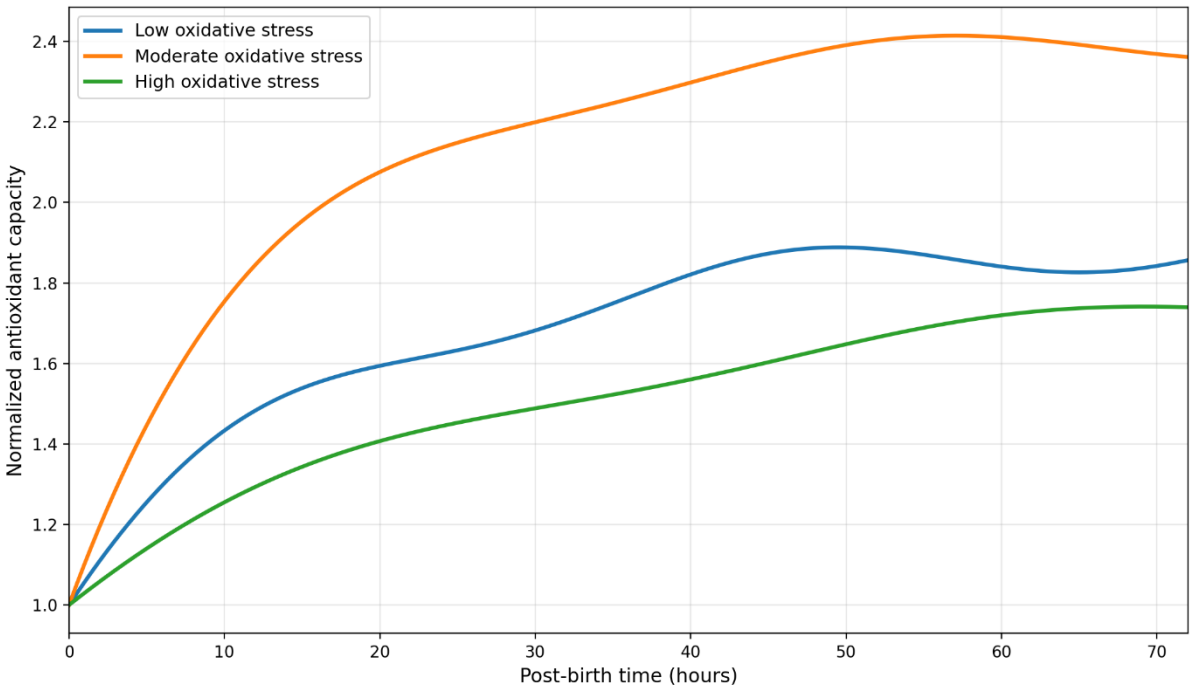

Figure 5. Time-resolved neonatal redox adjustment model (0–72 h post-birth)

Neonates with low to moderate oxidative stress display rapid increases in the upregulation of antioxidant enzymes with just a peak of activity after 24 to 36 hours. This rapid adjustment demonstrates the presence of functional redox sensing and regulatory mechanisms. In contrast, late and attenuated responses are observed in the high oxidative stress group. They also show slower increases in antioxidant activity and do not reach comparable levels of activity by 72 hours. This lag is indicative of chronic oxidative stress and a poor ability to compensate, predisposing these neonates to a longer time of physiological instability. The response is not instantaneous but rather evolving and the figure demonstrates this complex yet early stage of neonatal redox developments.

Table 2 summarizes the clinical adaptation metrics stratified by the degree of oxidative stress. For the neonates, oxidative stress was assessed via the cord blood redox signature, after which they were classified as low, moderate, and high oxidative stress. Table 2 includes the Apgar score, duration until the stabilization of oxygen saturation, and the occurrence of intermittent episodes of Thermoregulatory Unstable Equilibrium (TUE), along with certain in-table statistical comparisons. In line with Figures 4 and 5, the moderate oxidative load group still continues to show the most favorable adaptation profile, in particular with Apgar score with a greater value and a shorter duration until stabilization. In contrast, the high oxidative stress group has a greater delay in stabilization of oxygen saturation and a greater occurrence of episodes of thermoregulatory imbalance, while the low oxidative stress group has an intermediate profile.

Table 2. Clinical adaptation metrics stratified by oxidative stress burden

Oxidative stress burden group	Apgar score (5 min)	Oxygen saturation stabilization time (min)	Incidence of transient thermoregulatory instability (%)
Low oxidative load	8.6 ± 0.5	9.4 ± 2.1	11.8
Moderate oxidative load	**9.1 ± 0.4†**	**6.8 ± 1.7†**	**6.3†**
High oxidative load	7.9 ± 0.6‡	14.6 ± 3.4‡	24.5‡

† $p < 0.05$ vs. high oxidative load
‡ $p < 0.01$ vs. moderate oxidative load

The data presented in figures 4 and 5 and in table 2 correlate to reinforce the relationship between the degree of oxidative stress and neonatal adaptation. However, the relationship is more complex than a simple line connection, as the results indicate there is a redox window that is more favorable for the physiological changes to occur. Without the combined approach of stratified and visual analyses, the relationship may have gone unnoticed.

In addition to examining single biomarkers and outcomes, Figure 6 elaborates a network view of redox regulation and shows how the markers of oxidative damage and antioxidant enzymes interact. The graph-theoretic representation shows separate hubs for ROS and antioxidant enzymes, while the edge weights and the margins indicate the strength of the connection based on correlation and multivariate dependence. Antioxidant enzymes, including SOD and CAT, are identified as important central nodes, as they are highly interconnected to several oxidative markers, signifying their pivotal role in redox balance. In contrast, the group characterized by high oxidative stress displays a higher clustering of oxidative damage markers, suggesting the network lacks robustness, and the buffering capacity is low due to the absence of strong connections to antioxidant enzymes.

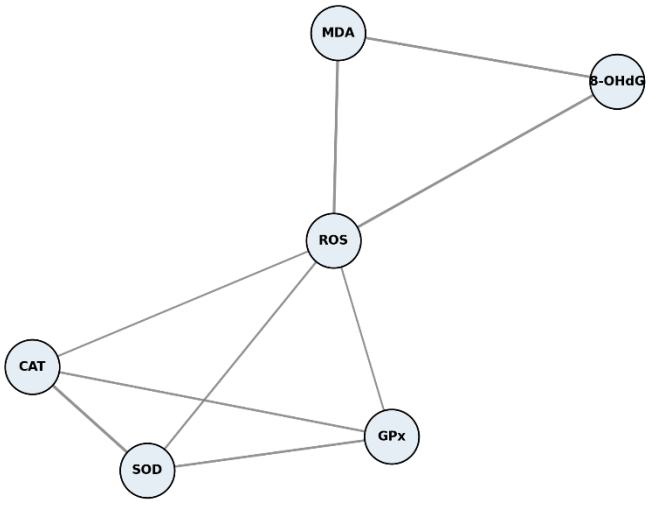

Figure 6. Network-level interaction map of oxidative stress and antioxidant pathways

This network perspective further complements the temporal and outcome-based analyses by conceptualizing the neonatal redox status as an interactive system, as opposed to a simply collection of discrete, independent variables. The structural differences in Figure 6 serve as a mechanistic perspective for the prolonged recovery

trajectories in Figure 5 and the adverse adaptation indices in Table 2. Antioxidant networks that are structurally well-connected allow neonates to redistribute the oxidative load.

Lastly, Figure 7 shows a predictive attempt by showcasing a classification model for the risk of neonatal adaptation based on redox profiles from cord blood. Using multivariate redox characteristics, the visualization, presented as a decision boundary or ROC surface, shows a distinct differentiation of outcomes of adaptation from the lows and the highs of risk. The output of the model is real analytic diagnostics rather than an abstract curve of performance, affirming the analytics value in practice. The model's capability to differentiate adjustment risk based on cord blood redox signatures aligns to a main hypothesis of the study -- the claim that oxidative stress at birth contain prognostic value that is actionable.

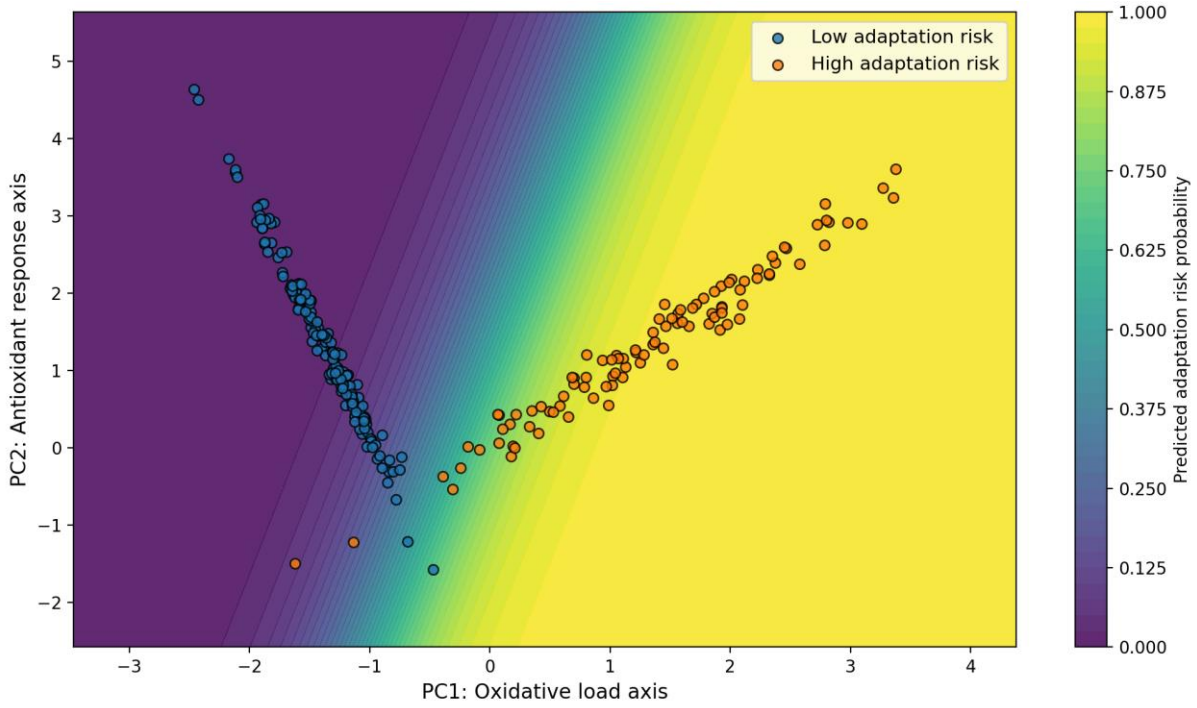

Figure 7. Predictive classification model for neonatal adaptation risk based on cord blood redox profile

The integrated analysis in this section crystallizes the link between the signatures of oxidative stress and early neonatal adaptation. Together, Figures 4 through 7 and Table 2 provides evidence that the redox balance at birth impacts the immediate physiological response through a non-linear, time-dependent, and network-mediated process. The biochemical, physiological and predictive evidence strengthens the argument that stress oxidative is a significant factor of neonatal transition rather than an insignificant byproduct of the birth process.

5. Conclusion

The study shows that the oxidative stress signatures in umbilical cord blood analysis are documented in the scientific literature and describe a structured and meaningful biological snapshot of the neonatal transition from intrauterine to extrauterine life. The signatures in the coordinated patterns of oxidative damage and antioxidant

defenses are not reflective of nonspecific biochemical noise. Instead, they represent early physiological adaptations. The integration of multivariate clustering, network-level interactions, and temporal adjustment dynamics reinforces the interpretation of cord blood redox profiles as adaptive biomarkers that differentiate neonates functioning within a healthy oxidative range from those with negative dysregulated responses at birth.

The documented relationship between oxidative stress burden and early neonatal adaptation outcomes reinforces the positive clinical application of redox profiling in a perinatal context. Stratification on the basis of cord blood oxidative signatures aligned with the differences in Apgar scores, oxygen stabilization, and thermoregulation, demonstrating the early potential of these biomarkers for risk stratification. The relationships were non-linear, indicating that the approach taken needs to avoid oversimplified interpretations of oxidative stress as “high versus low” and to embrace metrics where physiological redox balance is considered as a determining factor of successful neonatal adaptation.

From a forward-looking perspective, the findings support the incorporation of cord blood redox biomarkers into initial clinical decision pathways for the identification of neonates with potential risks for delayed physiological stabilization. Integrated assessment of biomarkers with multivariate and predictive analytics, as exemplified in this work, affords a means to assess early risk and tailor monitoring strategies without added clinical overload. By situating the assessment of neonatal adaptation in redox biology, this work demonstrates a mechanism for refining early interventions within the parameters of evidence-based medicine.

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