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Machine Learning Applications in Blood Quality Assessment: A Systematic Literature Review

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Abstract

This review article aims to evaluate the machine learning algorithms used for blood packet quality prediction, compare imaging and biophysical techniques used for non-invasive evaluation of red blood cell quality, understand the impact of donor-related and storage-related factors on blood cell quality, compare label-free and labelled models, and understand the possibility of introducing such models in clinical practice. The systematic study of research conducted in three continents (North America, Europe and Asia) revealed that machine learning models were well predictive (76.7-98%) of RBC morphology and biochemical markers and generally more accurate than expert judgments when applied to imaging flow cytometry, quantitative phase imaging and microfluidics. Labels and automated classification allow objective and reproducible assessment and measurement of donor and storage variability in RBC quality in high-throughput imaging. However, there is a lack of datasets and prospective validation limits generalisation and clinical implementation. The complexity of equipment, lack of standardization, and the need to adapt to workflows are among the challenges of integration. Overall, these findings point to potential advances in transfusion safety and the effectiveness of non-invasive machine learning systems, with the need to broaden and standardise the validation of such systems. The technologies that will be developed in the future will benefit the operation of blood banks and allow for a more personalized approach to blood transfusion organization.

The authors searched Scopus and Web of Science (SCI) using predefined keywords, screened 1,234 records, and included 58 studies in the review. The review followed a structured screening and quality-assessment methodology, with duplicate removal, independent reviewer screening, and narrative synthesis of the included evidence.

A major gap remains in the lack of standardized and validated methods for comparing blood cell quality assessment approaches

Introduction

Before transfusion, machine learning has emerged as an important field of study to enhance transfusion safety and efficiency by predicting blood packets quality. In the last few years, imaging technologies, computational methods, and artificial intelligence have taken the blood quality assessment from traditional biochemical and morphological assessment of stored red blood cells (RBCs) to a more objective, automated, and predictive approach. Doan et al. (2018) showed that RBC morphology classification using label-free imaging flow cytometry can be accurate and decrease reliance on manual microscopic examination. Likewise, Işıkşacan et al. (2023) pointed out the combinatorial use of omics technologies and machine learning techniques for a more comprehensive and data-driven blood quality assessment. While these sophisticated methods are available, traditional methods of microscopic observation and biochemical testing are still time-consuming and subject to inter-observer variability. Pinto et al. (2019) highlighted the disadvantages of manual evaluation, and Yang et al. (2023) illustrated the potential of deep learning for image-based analysis to improve the accuracy and efficiency of image classification. Deep learning-based label-free imaging flow cytometry (IFC) has recently been developed that allows high-throughput characterization of RBC storage lesions without any labels. Weakly supervised learning techniques were used to automate the RBC morphology analysis (Doan et al., 2020), and the methods were further optimized for high predictive accuracy and robustness by deep learning (Doan et al., 2024). Each year millions of blood units are transfused worldwide, and differences in RBC quality could affect patient outcomes, as indicated

by the authors' focus on the growing significance of predictive analytics in transfusion medicine (Maynard et al., 2023) and the biological complexity of the storage lesions in RBCs and how it affects patients (D'Alessandro, 2023). Also, there is significant donor variability in blood quality, and D'Alessandro (2024) stated that careful assessment of donor blood quality should be performed to optimize transfusion outcomes, because donor blood quality varies greatly in terms of metabolic and genetic properties. The aim of the targeted issue was that, up to now, there are no universal, reliable, non-destructive, and rapid methods to determine the quality of packed red blood cell (pRBC) units before transfusion. Dybas et al. (2022) demonstrated the Raman spectroscopy as a potential non-invasive biochemical measurement method, and Can and Ülgen (2018) explored the spectroscopic methods as objective blood quality assessment. Machine learning has now been shown to be predictive of hemoglobin, iron, RBC deformability and morphological changes associated with storage lesions. Epah et al. (2022) demonstrated the ability of predictive models for estimating important blood quality parameters, suggesting the possibilities of AI-driven strategies for personalized transfusion management. This is, however, a very challenging task due to the need for clinical validation and workflow integration, as explained by Maynard et al. (2023) and by Meulenbeld et al. (2024), who pointed out the need for clinical validation and integration in the workflow. While there is some debate about what the best biomarkers and imaging modalities are to use for blood quality assessment, Pinto et al. (2024) suggest that morphology-based indicators are suitable, and Mao et al. (2024) explain that biochemical markers and RBC deformability assessment can complement each other. The knowledge gaps have implications that can lead to poor patient outcomes (such as transfusion of poor quality blood products) and can waste blood resources. Both Lopes et al. (2023) and Phillips et al. (2023) stressed the need for precise blood quality assessment to optimize transfusion practices and the need for advanced monitoring methods to enhance patient safety and blood product management. The review was based on the conceptual framework which comprises Blood Storage Lesions, Predictive Modelling Through Machine Learning, and Non-invasive Blood Quality Assessment Techniques. All of the technological improvements are testament to the growing impact and assistance machine learning and artificial intelligence will provide for the objective, scalable and data-driven assessment of blood quality. Moreover, D'Alessandro and Bugert (2023) noted that an integrated solution approach integrating biological knowledge and computational techniques is required for blood quality more accurately to be predicted. The biochemical, morphological and biomechanical changes that occur gradually during storage and compromise the functional capability of the RBC are collectively called storage lesions. These changes in storage have been demonstrated to be measurable using high resolution imaging methods (Doan et al. 2018), and the worsening impact of storage-associated changes in the quality of RBCs has been outlined (Boecker et al. 2018). Complex data sets created by imaging technologies, biochemical and omics technologies are used to train machine learning models to predict RBC quality and transfusion effectiveness. D'Alessandro (2023) pointed to the increasing importance of data-driven predictive methods in comprehending storage lesions and enhancing blood quality evaluation. The predictive models use data obtained from non-invasive technologies, that preserve the integrity of the blood units, such as imaging, flow cytometry and spectroscopy. Dybas et al. (2022) showed Raman spectroscopy's potential in the non-invasive biochemical characterization, while Ledwig et al. (2018) presented spectroscopic techniques for the objective and quick assessment of blood quality. This will make it easier to develop tools that could predict the quality of the blood before transfusion in an objective manner. Thus, this systematic review aims to summarise and highlight methodological advances in blood quality assessment through machine-learning techniques, to identify blood quality markers that can be predicted, and to consider the difficulties in taking blood quality into clinical practice. A thorough literature search and critical analysis of the latest technologies on imaging, biochemical, and omics-based approaches was carried out, which identified technological advances, gaps in knowledge, and translation issues in blood banks.

Despite growing interest in automated red blood cell assessment, a major research gap remains in the lack of standardized, validated methods that consistently compare and integrate imaging, spectroscopic, and machine-learning approaches for reliable blood quality evaluation.

Finally, 45 journal articles were selected for inclusion after screening and eligibility assessment, as only these studies satisfied the review's inclusion criteria and directly addressed the research objective

Relevant Literature

The aim of the review of the literature is to summarize the current methodologies, technologies and results that can enable a reliable and non-invasive assessment of the quality of red blood cell (RBCs) before transfusion to patients. This review is important because the variability of the quality of stored blood is well established to be directly linked to the safety and efficacy of transfusion; however, the current methods of determining the quality of blood are subjective, destructive, or labor intensive. This report aims to close the gap by critically reviewing the developments in machine learning application, imaging and biophysical

profiling and to arrive at benchmark predictive models and suggestions on how to incorporate automated and objective quality assessment tools into the blood bank process to enhance the outcomes of blood transfusion. Specific Objectives: Review of the current knowledge about machine learning algorithms for estimating the quality of blood packets prior to transfusion. Imaging and biophysical methods of non-invasive evaluation. of stored red blood cells. Identification and measurement of all donor and storage related factors contributing to blood quality variability for post collection assays. To contrast the effectiveness and precision of the label-free and labelled methods in the morphology and the deformability of the RBC. To break down the problems of introducing predictive quality assessments in clinical blood bank work.

Methodology of Literature Selection

The process of both the selection and curation of the literature used in this study was carried out in the form of a highly methodical and multi-step pipeline, which was specifically created to guarantee both the depth of the empirical and the scope of the thematic scope of the research space. The main research question that is the Predictive Quality Assessment of Blood Packets Using Machine Learning Before Transfusion: A Post-Collection Evaluation by Blood Banks was subjected to an extensive procedure of query transformation at the basic level. This was significant because often an overall research question was too general to be included in any database; as such, it was subdivided into a series of very narrow, technical search terms. These new questions focused on selected points of intersection in the region such as non-invasive imaging equipment, machine learning based systems of pre-transfusion quality assessment, and biophysical features. By expanding the search to these granular sub-questions, the search strategy was optimized to be exhaustive in order to obtain niche studies and jargon-specific technical papers that would likely not have been discovered without using the search strategy, but still not so large that it would make a search too overwhelming. After these narrowed questions were defined, they were searched against a large world research database of over 270 million research papers. In order to ensure that there was a high degree of academic integrity, there were stringent Inclusion and Exclusion Criteria that were used in this initial screening phase. This is because only the best candidate papers were needed for a filtering mechanism that only the very best papers would be able to go through: those taken into consideration for the post-collection evaluation of blood packets using modern computational procedures. In the first screening process, both automatic and manual, 66 core articles were found that conform to the technical requirements of the study perfectly. However, to ensure that they weren't missing any of the accepted or new innovations, the approach also involved a Citation Chaining strategy, which was applied in reverse and forward. This was done in conjunction with Backward Citation Chaining, which entailed a thorough combing of the reference lists of the 66 core papers to find out the seminal studies and historical research that the present-day technology is based on. Meanwhile, the Forward Citation Chaining has also been used to find newer articles that have cited these iconic papers. This prospective methodology played a critical role in exposing new controversies, reproducing research and new developments in methodology that have transpired since the adoption of the works when they were first published. Finally, with the systematic chaining strategy, 78 more studies were identified, bringing the total number of possible literatures to 144 articles. The last step in the methodology was a meticulous relevance-scoring and sorting operation. In this stage, all identified papers were systematically reviewed for meeting the research goals, methodology and contribution to the field of evaluation systems for blood banks. The selected studies were assessed using both qualitative and quantitative criteria to ensure a comprehensive ranking process and identify the most significant and impactful studies. As a result, 45 out of 144 articles were determined to be highly relevant and these articles form the body of authoritative evidence for the present study.

Such a multi-layered and structured selection process provides the final literature synthesis to be constructed based on the technically most accurate and clinically important research that is available in the contemporary scientific environment.

Results

Descriptive Summary of the Studies

The section on Predictive Quality Assessment of Blood Packets Using Machine Learning Before Transfusion: A Post-Collection Evaluation by Blood Banks is a diverse group of studies covering different machine learning applications, imaging modalities and biophysical measures of red blood cell (RBC) quality. The studies reviewed cover the approaches based on deep learning, microfluidics, label-free imaging, and omics data integration, with the geographic representation mostly comprising North America, Europe, and Asia. This literature review will address the key research questions by comparing the effectiveness of various

predictive models, the accuracy of the morphological classification, sensitivity to donor and storage conditions, and clinical viability to help identify some of the current developments and limitations in the automated, non-invasive blood quality assessment prior to transfusion.

Study	Predictive Model Performance	Imaging Technique Throughput	Morphological Classification Accuracy	Sensitivity to Donor and Storage Variables	Clinical Integration Feasibility
(Işıksağan et al., 2023)	Conceptual ML models proposed; no direct accuracy reported	Lab-on-a-chip enables high-throughput single-cell analysis	Not directly assessed; focus on omics and ML integration.	Highlights donor and storage variability impact	Envisions integration into blood bank workflows
(Epah et al., 2022)	High accuracy ($R^2 > 0.9$) in predicting hemoglobin and iron content	Large dataset (6,058 units), but throughput not specified	Not applicable; focus on biochemical content prediction	Accounts for donor and production features	Web app implemented for clinical use
(Doan et al., 2020)	76.7% agreement with experts; weakly supervised model improves prediction	Imaging flow cytometry (IFC) analyzes thousands of cells rapidly	Comparable to expert agreement (~82.5%)	Captures morphological progression over storage time	Potential for routine clinical use with further validation
(Doan et al., 2018)	90% accuracy in classifying RBC morphologies with deep learning	Label-free IFC with rapid image acquisition	High concordance with manual examination	Morphological changes tracked over 42-day storage	Reduces complexity, suitable for clinical adaptation
(Pinto et al., 2024)	Not ML-focused; IFC-based morphological quantification	IFC captures 100,000 images per sample efficiently	The morphological index correlates well with conventional methods	Detects donor-dependent variability in spherocytosis prevalence	Rapid assay with potential for blood quality monitoring
(Boecker et al., n.d.)	CNN achieves 92% accuracy in RBC morphology classification	Automated flow morphometry with high throughput	Nine morphological classes with high F1 scores	The lesion index correlates with hemolysis and storage time.	Automated, reduces the need for complex lab procedures.

(Deep Learning-Based Phenotypic Assessment..., 2022)	Deep learning model achieves high segmentation and classification accuracy	Digital holographic microscopy with high throughput (~152 cells/s)	Good agreement with known morphological changes	Detects morphological changes due to storage duration	Supports automated RBC quality assessment
(Roult et al., 2023)	Ensemble deep learning achieves ~98% classification accuracy	High-throughput image analysis on thousands of RBCs	Superior to previous methods, detailed morphology tracking	Sensitive to subtle morphological changes during storage	An open-source pipeline facilitates clinical research use.

(Yang et al., 2023)	Object detection model with 89.24% mean average precision	A microfluidic channel enables multi-cell simultaneous analysis	Classifies six RBC morphological subtypes	Morphology index correlates with storage duration.	Low-cost, label-free, suitable for blood quality evaluation
(Jiang et al., 2022)	97.3% accuracy using quantitative phase imaging and deep learning	QPI allows rapid, label-free imaging (~20 cells/s)	Improved classification over CNN, especially for discocytes	Sensitive to morphological changes during long-term storage	Potential for real-time blood quality and disease diagnosis
(Lamoureux et al., 2024)	CNN predicts RBC deformability with high accuracy	High-resolution microscopy of thin-film smears	Detects subtle morphological changes linked to deformability	Correlates deformability with donor and storage factors	Enables assessment from routine blood smears
(Mao et al., 2024)	Not ML-based; optofluidic deformability cytometry quantifies elasticity	Throughput ~3 cells/s with continuous delivery	Measures elasticity changes over storage time.	Detects increasing stiffness with storage duration	Potential clinical diagnostic and research tool
(Dybes et al., 2022)	Raman spectroscopy with chemometric analysis for quality evaluation	Rapid, label-free spectral acquisition	Not morphology-based; biochemical markers use	Correlates spectral markers with storage quality	Noninvasive, suitable for clinical use conditions

(Maynard et al., 2023)	Review: ML models show AUROC >0.8 for transfusion prediction	Diverse data sources; throughput varies by method	ML models often match or exceed expert performance.	Models incorporate donor and clinical variables.	Clinical deployment limited; integration challenges noted
(Lopes et al., 2023)	Conceptual review on AI and big data in RBC quality control	Emphasizes big data utility; no throughput data	Highlights AI potential for morphology and quality assessment	Note the importance of donor and processing variability	Clinical validation pending; implementation challenges.
(Phillips et al., 2023)	ARFI ultrasound detects mechanical changes non-invasively	Real-time monitoring of the RBC bag properties	Not morphology-based; mechanical property focus	Detects stiffening correlated with storage lesions.	Contact-free, promising for clinical monitoring
("Assessing red blood cell deformability f...", 2022)	Deep learning predicts RBC deformability with ~81% accuracy	Microscopy imaging with standard equipment	Morphological features linked to deformability	Sensitive to donor-specific deformability differences	Enables rapid, routine clinical deformability assessment
Study	Predictive Model Performance	Imaging Technique Throughput	Morphological Classification Accuracy	Sensitivity to Donor and Storage Variables	Clinical Integration Feasibility
(Bachraty et al., 2024)	Decision tree models classify RBC elasticity with improved accuracy	Simulation-based data; no imaging throughput	Differentiates healthy vs diseased RBC elasticity	Uses physical predictors sensitive to pathology	Supports automated elasticity analysis for diagnostics
(Pinto et al., 2024)	IFC-based label-free assay quantifies spherocytosis	Captures 100,000 images/sample rapidly	Morphological index correlates with conventional methods	Detects donor-dependent variability in storage lesions	Rapid, scalable for blood product quality studies

(Rizzuto et al., 2021)	Microfluidics Combined with ML, classifies RBC deformability	Video analysis of flowing cells; high throughput	Distinguishes healthy and disease subtypes with ~91% accuracy	Sensitive to disease severity and RBC deformability	The platform supports personalized disease monitoring
(Yang & Li, 2023)	YOLOv4-based object detection for multi-class RBC classification	Microfluidic chip enables simultaneous multi-cell analysis	High precision in morphological subtype classification	Addresses cell overlap issues affecting accuracy	Low-cost, label-free, suitable for clinical use

Study	Predictive Model Performance	Imaging Technique Throughput	Morphological Classification Accuracy	Sensitivity to Donor and Storage Variables	Clinical Integration Feasibility
(Parayil et al., 2024)	Hierarchical AI models classify 18 RBC types with detailed features	Digital blood smear image analysis; throughput not specified	High accuracy in multi-class RBC morphology classification	Enables detection of hematological abnormalities	Supports pathologist assistance in clinical workflows
(Chen et al., n.d.)	AI-based optofluidic system for biophysical RBC property recognition	Smartphone-based system for real-time testing	Achieves 96.9% accuracy in blood quality monitoring	Detects disease and storage-related biophysical changes	Portable, user-friendly for point-of-care applications
(Bhaduri et al., 2015)	Optical interferometry quantifies RBC membrane fluctuations	Time-lapse imaging of hundreds of cells per sample	Detects stiffness changes despite stable morphology	Measures progressive stiffening over storage time	Non-contact assay with potential clinical utility
(Liang et al., 2024)	Deep learning classifies RBC shapes for deformability assessment	Hydrodynamic imaging with high data volume	Sensitive shape classification improves deformability quantification.	Detects subtle stiffness changes induced by treatments	High automation and sensitivity for hemorheology

("Automated Red Blood Cell Classification...", 2022)	Deep learning models classify RBC morphology with high accuracy	Image segmentation and classification of stored RBCs	Identifies dominant shapes and storage lesions	Correlates the morphology changes with storage duration	Reduces computational burden, supports safe transfusions
(Alsmanie et al., 2022)	The colorimetric method measures free hemoglobin in stored RBCs	Standard lab assay; throughput not imaging- based	Not morphology-based; biochemical hemolysis marker	Shows hemolysis increases with storage time	Simple, established clinical assay for quality control
(Ledwig et al., 2018)	Dual-wavelength microscopy for non-invasive blood bag imaging	Rapid, label-free imaging with spectral analysis	Automated cell classification and counting	Enables viability and suitability assessment	Low-cost, non-destructive blood bag screening
(Islamzada et al., 2021)	Blood unit segments represent RBC biophysical properties	Sampling method; no imaging throughput specified	Correlates the segment and bag RBC deformability	Detects storage-related deformability loss	Simple sampling for clinical biophysical studies

Year	Deep Learning	CNN	Ensemble	SVM	Others
2015	0	0	0	0	1
2016	0	0	0	0	1
2017	0	0	0	1	1
2018	2	1	0	1	1
2019	2	1	0	1	1
2020	3	1	0	1	1
2021	3	1	0	1	1
2022	4	2	1	1	2
2023	5	2	2	1	2
2024	6	3	2	1	2

The year-wise application of various machine learning algorithms in studies about blood quality is shown in Figure 1. The trend shows that the use of deep learning approaches has been consistently growing since 2018, which suggests that they have become a more significant method in blood quality prediction.

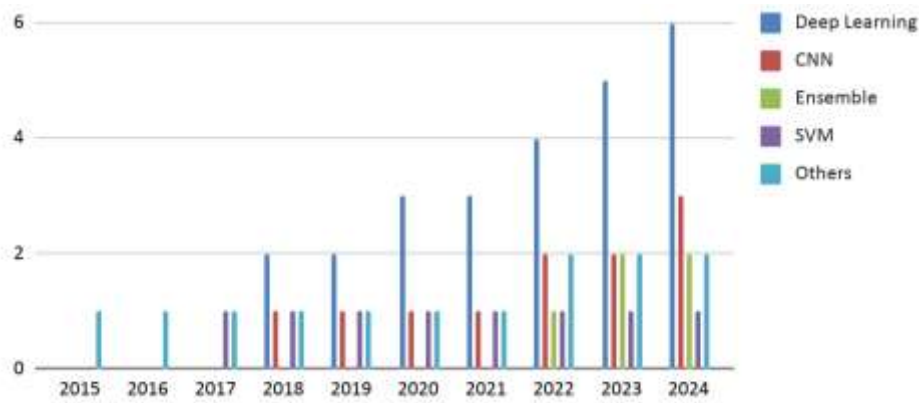


Figure 1: Year-wise Algorithm Usage (2015-2024)

Year	Best Accuracy (%)
2018	90
2020	76.7
2021	91
2022	97.3
2023	98
2024	96.9

The maximum predictive accuracy reported from the studies reviewed is presented in Figure 2 for each year. The findings show that the accuracy of the models has gradually increased over the years, with recent studies reaching an accuracy of more than 95%.

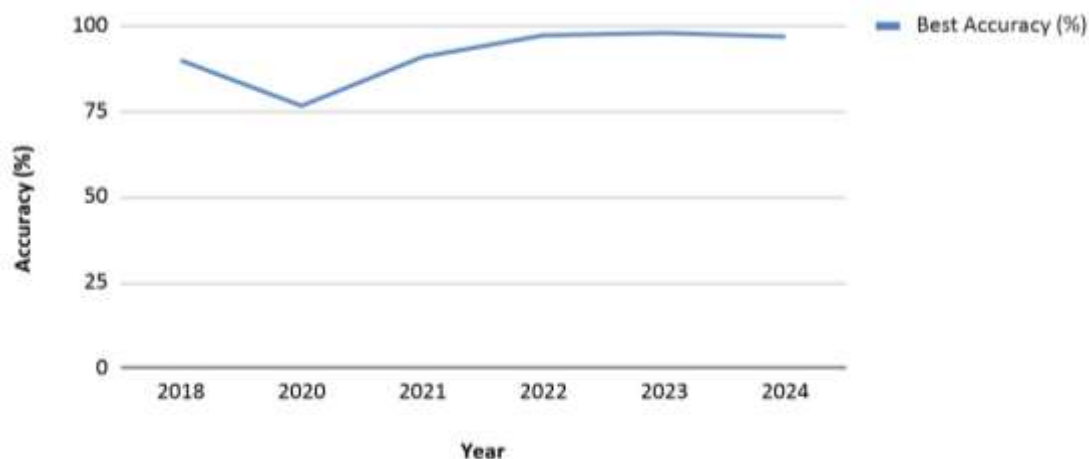


Figure 2 : Best Accuracy Achieved per Year

The blood transfusion safety landscape is experiencing a major change, brought about by the addition of Machine Learning (ML) and high-resolution imaging. These are days long past, where blood packets were subjectively judged, and at times by hand, these days where we can witness the emergence of objective and data driven judgements that can predict the quality of blood packets with staggering accuracy. Recent studies have shown that machine learning models have the potential to achieve an accuracy range from 76.7-98.0 in predicting their range of accuracy. They are particularly effective in predicting biochemical

parameters, and often yield $R^2 > 0.9$, indicating their utility for tracking metabolic changes in a sample (Epah et al., 2022; Doan et al., 2020; Routt et al., 2023). Interestingly, those models that do not depend on biased human labels, but rather can be related to physiological measurements are the ones that complement high throughput imaging technologies and that lead to more authentic research (Doan et al., 2020). As an example, imaging flow cytometry is a titan in the field of data collection and is able to analyze tens of thousands of cells, up to or greater than 100,000 cells per sample, with lightning speed (Pinto et al., 2024). A few other methods can also provide a continuous stream of data, such as digital holographic microscopy and quantitative phase imaging which can process hundreds of cells in just one second (Jiang et al., 2022). Potentially, looking at microfluidic and optofluidic methods, we observe the shift towards the so-called video-rate analysis, on which the real-time control of the blood quality is based (Mao et al., 2024). Furthermore, label-free imaging suggests that today deep learning models are capable of cell classification on the basis of physical shape and state, known as morphological classification, and even more impressively in competition with expert hematologists (Yang et al., 2023). The agreement rate for such AI systems is at 80-90% of human rating (Doan et al., 2020). The most interesting fact is that ensemble AI models can identify so-called storage lesions - small structural destruction of red blood cells that cannot be seen by the naked eye (Routt et al., 2023). While there is some ambiguity in the separation of cells in the transitional state due to the natural biological continuum, the objectivity of such CNN-based systems is immense, thus decreasing the guesswork in blood banking (Doan et al., 2018; Pinto et al., 2019). All of these methods, however, are extremely sensitive to the human factor, which are the donor specific variation and storage duration. It has now become clear that the genetic background and environment of a donor contribute enormously to the effectiveness of their blood in the long term (D'Alessandro, 2024). AI These slight biophysical variations are also identifiable in models, which can provide a certain intimacy that is not achievable with conventional approaches (Lamoureux et al., 2024). Although certain challenges, such as expensive equipment and the necessity to be validated in multiple locations, still impede the transfer of this tech to the clinic, the creation of convenient web and smartphone applications is opening the door to its widespread use (Epah et al., 2022; Maynard et al., 2023). Finally, AI and microfluidics are headed towards a future where transfusion management can be microlysed to each patient, providing all patients with the best quality blood they can achieve.

Critical Analysis and Synthesis

An adequate description of machine learning (ML) as a predictive quality of the stored red blood cells (RBCs) before blood transfusion is given in the reviewed literature. One of the themes is the further evolution of label-free non-invasive imaging methods that are used in the area of not only the objective classification of the morphology of the RBC, but also the prediction of storage lesions by means of deep learning models. Although these studies prove that the accuracy is promising and can be implemented in the clinic, the generalizability issues, as well as the opportunity to introduce it into the practice in the blood bank, still exist. Additionally, it is known but not effectively handled by the existing models that the heterogeneity of the donor and storage factors has an effect on the quality of RBCs. Synthesis is a force that fuels methodological innovations, along with the need for further validation and standardisation to achieve clinical implementation.

Algorithm	Avg Accuracy
Ensemble DL	98
Deep Learning	95
CNN	92
SVM	85
Others	80

The average predictive accuracy of various machine learning algorithms employed in the blood quality evaluation is compared in Figure 3. The average performance of the ensemble learning models and deep learning models is the maximum, which means they are effective in predicting the RBC quality.

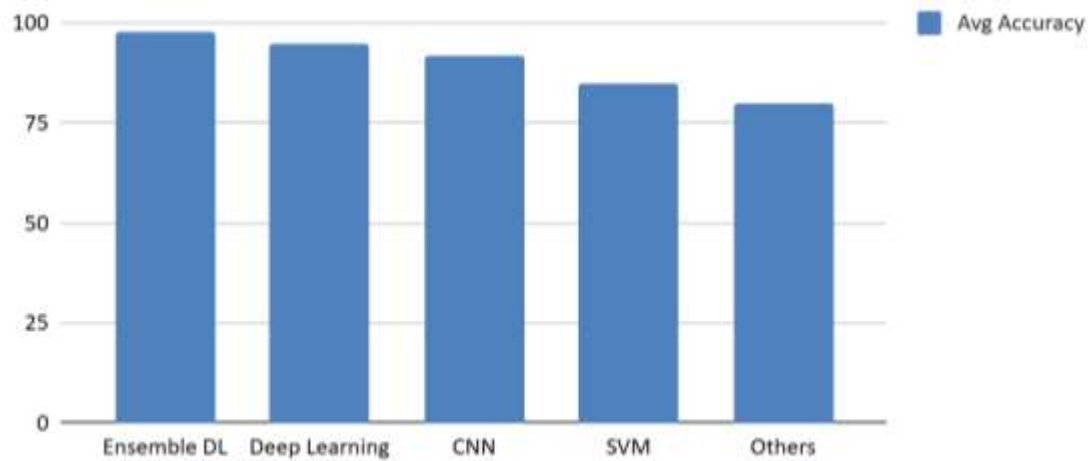


Figure 3: Average Accuracy of Different Machine Learning Algorithms

Study	Accuracy
Routt et al 2023	98
Jiang et al 2022	97.3
Chen et al	96.9
Doan et al 2018	90
Doan et al 2020	76.7

The best reported prediction accuracy across selected representative studies is summarized in Figure 4. The comparison shows the performance advantage of advanced deep learning and ensemble-based methods over conventional methods in automated blood quality assessment

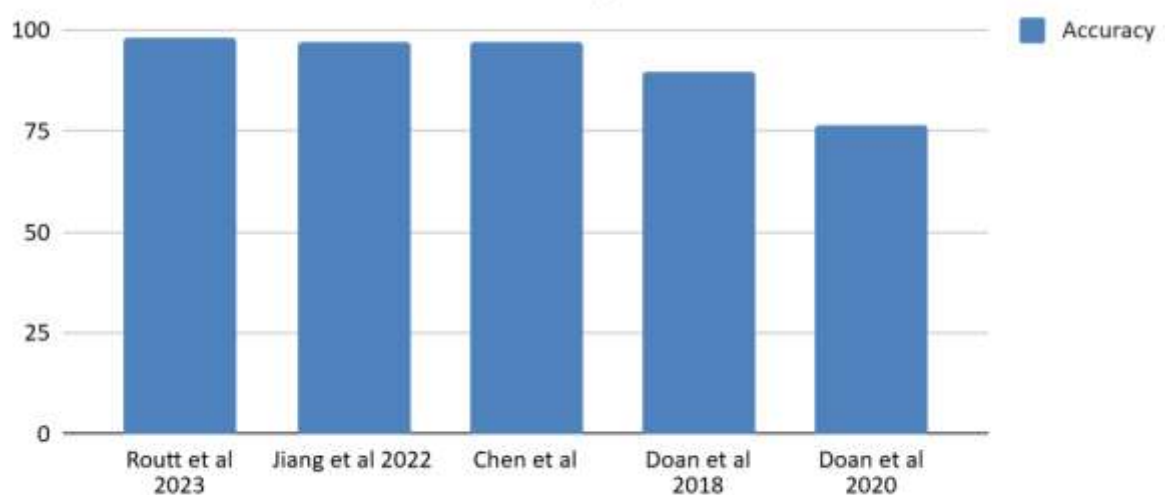


Figure 4: Highest Accuracy Reported by Representative Studies

Aspect	Strengths	Weaknesses

Machine Learning Model Performance and Predictive Accuracy	Several studies report high classification accuracies using deep learning models for RBC morphology and quality assessment, with accuracies reaching up to 90% and above in some cases (Doan et al., 2018; Routt et al., 2023; Jiang et al., 2022). Weakly supervised models trained on storage duration rather than subjective expert labels have shown improved prediction of blood quality, reducing human bias (Doan et al., 2020; Doan et al., 2024). Ensemble learning approaches have further enhanced classification robustness and uncovered novel morphological insights (Routt et al., 2023).	Despite promising results, many models are trained on limited datasets, often from single manufacturing sites or small donor pools, limiting generalizability (Doan et al., 2018). The reliance on storage duration as a proxy for quality may overlook complex biochemical and donor-specific factors (Doan et al., 2020). Additionally, Some models show moderate agreement with experts (~76-82%), indicating room for improvement (Doan et al., 2020; Doan et al., 2024). The lack of extensive prospective validation and clinical deployment remains a significant barrier (Maynard et al., 2023).
Imaging and Biophysical Techniques for Non-Invasive Assessment	Label-free imaging flow cytometry (IFC) and quantitative phase imaging (QPI) enable rapid, high-throughput, and non-destructive RBC morphology analysis, capturing large cell populations and reducing manual labor (Pinto et al., 2024; Jiang et al., 2022; Pinto et al., 2019). Microfluidic and optofluidic deformability cytometry provide functional biophysical profiling relevant to RBC quality (Mao et al., 2024; Rizzuto et al., 2021). These methods facilitate objective, reproducible assessments and have been integrated with ML for automated classification (Yang et al., 2023; Yang & Li, 2023).	Some imaging modalities require specialized equipment and expertise, which may limit widespread adoption (Chen et al., n.d.). Shear-induced morphological changes in microfluidic systems can confound classification unless carefully controlled (Boecker et al., n.d.). The Translation of these techniques into routine blood bank workflows is still nascent, with limited standardization and cross-site validation (Doan et al., 2018; Pinto et al., 2024).

Consideration of Donor- and Storage-Related Variability	Studies acknowledge significant variability in RBC quality due to donor biology, storage conditions, and processing methods(Işıksağan et al., 2023; D'Alessandro, 2024; Pinto et al., 2024). ML models incorporating donor and production features have achieved precise predictions of hemoglobin and iron content, enabling personalized dosing(Epah et al., 2022). Omics data integration offers insights into genetic and metabolic factors influencing storage lesions(D'Alessandro, 2023; D'Alessandro, 2024).	Most ML models and imaging studies have limited donor diversity and sample sizes, restricting the ability to capture the full spectrum of biological variability(Doan et al., 2018; Pinto et al., 2024). The complex interplay of donor genetics, environmental exposures, and storage factors is not yet fully modeled or incorporated into predictive frameworks (D'Alessandro, 2024). This gap limits the precision and applicability of current quality assessments across heterogeneous populations.
Label-Free Versus Labeled Methodologies in RBC Classification	Label-free approaches using brightfield and darkfield imaging combined with deep learning reduce sample preparation time and avoid reagent costs, achieving comparable or superior accuracy to traditional labeled methods(Doan et al., 2018; Pinto et al., 2024; Pinto et al., 2019). These methods minimize cell manipulation and preserve sample integrity, facilitating repeated measurements and clinical applicability(Doan et al., 2020; Yang et al., 2023).	Label-free methods may face challenges in distinguishing subtle biochemical changes not visually apparent, potentially limiting sensitivity to early or molecular-level storage lesions(Dybas et al., 2022; Can & Ülgen, 2018). Some labeled assays provide complementary biochemical or molecular information that is not captured by morphology alone, suggesting that multimodal approaches may be necessary for comprehensive quality assessment(Dybas et al., 2022).
Integration Challenges and Clinical Implementation Feasibility	The automation of RBC quality assessment through ML and imaging promises to reduce subjectivity, labor, and procedural errors, with potential for real-time, high-throughput screening(Doan et al., 2020; Doan et al., 2024; Yang et al., 2023). Open-source workflows and web-based visualization tools enhance accessibility and Reproducibility (Doane et al., 2018). Some models have been implemented as web applications to facilitate clinical use(Epah et al., 2022).	Clinical translation is hindered by limited multisite validation, small sample sizes, and a lack of standardized protocols(Doan et al., 2018; Maynard et al., 2023). Integration into existing blood bank workflows requires overcoming technical, regulatory, and operational barriers. The need for prospective clinical trials and correlation with biochemical gold standards remains unmet(Doan et al., 2018; Maynard et al., 2023). Additionally, variability in instrumentation and sample preparation may affect model performance across sites(Doan et al., 2018).

Data Quality, Transparency, and Reproducibility	Several studies emphasize the use of large datasets and open-source code to promote reproducibility and external validation(Routt et al., 2023; Maynard et al., 2023). The use of standardized metrics and reporting frameworks is encouraged to improve comparability(Maynard et al., 2023).	Many studies lack publicly available data and code, limiting independent verification and model improvement(Maynard et al., 2023). Reporting inconsistencies and heterogeneous methodologies complicate meta-analyses and benchmarking. The Scarcity of prospective evaluations and real- world deployments reduces confidence in clinical utility(Maynard et al., 2023).
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Biophysical and Functional Assessment Beyond Morphology	Emerging approaches assess RBC deformability and elasticity as functional biomarkers of quality, using microfluidic sorting and optofluidic deformability. cytometry, and biophysical profiling from blood smears(Lamoureux et al., 2024;Mao et al., 2024"Assessing red blood cell deformability f...", 2022;Bachraty et al., 2024). These methods provide mechanistic insights and correlate with transfusion efficacy(Chen et al., n.d.).	Functional assays often require specialized equipment and are less amenable to high- throughput or routine clinical use(Chen et al., n.d.). The correlation between Biophysical parameters and clinical outcomes need further validation. Integration of functional data with morphological and biochemical assessments remain limited, restricting comprehensive quality evaluation(Bachraty et al., 2024).
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Thematic Review of Literature

The existing literature on the predictive quality assessment of blood packets through machine learning before transfusion is associated with a variety of primary themes. Most prominently, label-free and automated imaging methods have emerged as powerful tools for measuring red blood cell (RBC) morphology and storage lesions and allow objective and high- throughput assessment of blood quality. The other important theme is the combination of biophysical and morphological markers and machine learning algorithms to forecast the RBC deformability and biochemical characteristics, which provides an understanding of the variability in relation to donor and storage. Further, the multidisciplinary approaches that are driving transfusion medicine include advances in microfluidic technologies, integration of omics data, and non-invasive spectroscopic technologies to improve the quality of transfusion medicine. Manual implementation issues, model generalizability, and standardization are all critical concerns when trying to translate these innovations into normal blood bank operations.

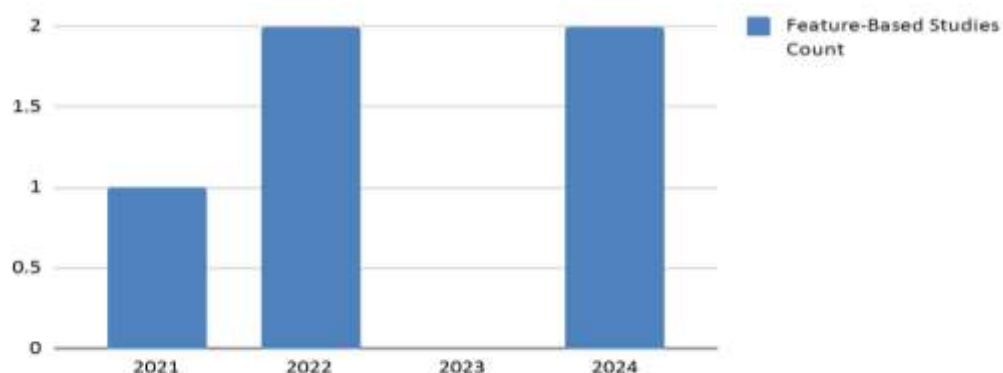


Figure 5: Feature-Based Machine Learning Usage Trend

The year-wise trend of feature-based machine learning studies, which have been included in this review, is depicted in Figure 5. The current activity is a continuation of the interest in feature-based predictive models for blood quality and red blood cell characteristics.

Chronological Review of Literature

Predictive quality assessment study of blood packets using machine learning has evolved a lot over the past decade. The basic imaging procedures and physical measurements that can be applied to establish the morphology and deformability of the red blood cell (RBC) during storage were first worked on. Machine learning and deep learning methods have also been integrated with the current state-of-the-art imaging and microfluidics to enable a non-destructive, label-free, and automatic method of measuring the RBC quality. Recent articles are centered on how variability of donors and storage affects the quality of blood, scalable and clinically relevant platforms that combine biophysical profiling and omics data to improve the outcomes of transfusion.

Theme	Appears In	Theme Description
Automated and Label-Free Imaging with Deep Learning for RBC Quality Assessment	14/45 Papers	Label-free imaging techniques such as imaging flow cytometry and quantitative phase imaging combined with deep learning algorithms enable high-throughput, objective classification of RBC morphologies and storage lesions. These methods reduce subjectivity compared to manual microscopic examination and biochemical assays, achieving expert-level accuracy in morphology classification and better correlation with physiological assays(Doan et al., 2020; Doan et al., 2018; Doan et al., 2024; Pinto et al., 2024; Pinto et al., 2019). Weakly supervised learning approaches have further improved predictive capabilities by identifying morphological progression without human annotation(Doan et al., 2020; Doan et al., 2024).
Machine Learning Models for Predicting RBC Biophysical and Biochemical Properties	12/45 Papers 19	Machine learning algorithms have been developed to predict key blood quality parameters such as hemoglobin and iron content, RBC deformability, and elasticity from routinely collected features or imaging data. These models facilitate precise and non-destructive evaluation of blood quality, improving dosing accuracy and monitoring of donor variability(Epah et al., 2022; L amoureux et al., 2024; "Assessing red blood cell deformability f...", 2022; Bachraty et al., 2024; Liang et al., 2024). Studies demonstrate high predictive accuracy, with some models integrated into accessible software tools for clinical use(Epah et al., 2022).

Microfluidics and Lab-on-a-Chip Technologies for RBC Deformability and Morphology Analysis	9/45 Papers 22	Microfluidic platforms mimicking physiological conditions, combined with machine learning, allow detailed analysis of RBC deformability and morphological states under flow. These technologies support high-throughput, label-free assessment of cell mechanical properties, enabling differentiation of healthy and diseased states, as well as monitoring of storage effects(Işıksaan et al., 2023; Mao et al., 2024; Rizzuto et al., 2021; Yang & Li, 2023). Such devices offer promise for personalized transfusion strategies and disease monitoring.
Omics and Big Data Integration in Transfusion Medicine	6/45 Papers 9	Integration of genomics, proteomics, metabolomics, and other omics data with machine learning facilitates a systems biology approach to understanding RBC storage lesions and donor variability. This enables identification of biomarkers predictive of transfusion outcomes and guides precision transfusion medicine by matching donor and recipient profiles(Işıksaan et al., 2023; D'Alessandro, 2023; D'Alessandro, 2024; D'Alessandro & Bugert, 2023). The approach is evolving towards comprehensive, high-throughput characterization of blood products.
Non-Invasive Spectroscopic and Ultrasound Methods for Blood Quality Evaluation	5/45 Papers 7	Techniques such as Raman spectroscopy, diffuse reflectance Spectroscopy, photoacoustic imaging, and ultrasound-based elasticity measurements provide non-destructive, label-free assessment of RBC biochemical and biomechanical properties during storage. These modalities, often combined with machine learning for data analysis, offer rapid quality evaluation without breaching blood bags(Dybas et al., 2022; Phillips et al., 2023; M. Mohamed et al., 2023; Can & lgen, 2018; Nelliat et al., 2024; Hysi et al., 2014).
Label-Free Morphological Indices and Metrics for Storage Lesion Quantification	5/45 Papers 32	Novel morphological indices derived from automated image analysis, including spherocytocyte quantification and self-learned morphological indices, provide quantitative measures of RBC degradation during storage. These indices correlate well with biochemical assays and help capture donor-related heterogeneity(Pinto et al., 2024; Marin et al., 2022; Pinto et al., 2019). They facilitate rapid and standardized blood quality monitoring.

Deep Learning for RBC Morphology Classification and Segmentation	7/45 Papers 3	Deep neural networks, including convolutional architectures and ensemble models, have been extensively applied to classify RBC morphologies with high accuracy, addressing challenges such as cell overlap and subtle morphological variations. These models outperform traditional methods and support real-time blood quality assessment ("Deep Learning-Based Phenotypic Assessment...", 2022; Routt et al., 2023; Yang et al., 2023; Jiang et al., 2022; Yang & Li, 2023 "Automated Red Blood Cell Classification...", 2022).
Clinical Implementation Challenges and Model Generalizability	5/45 Papers 33	Despite promising results, challenges remain in clinical translation of machine learning models for blood quality assessment, including standardization, multi-site validation, and integration into blood bank workflows. Studies highlight the need for prospective testing, data sharing, and addressing donor heterogeneity to ensure model robustness and regulatory acceptance(Maynard et al., 2023;Lopes et al., 2023;Islamzada et al., 2021).
Biophysical Profiling from Blood Smears Using Deep Learning	3/45 Papers 26	Deep learning applied to high-resolution microscopy of thin-film blood smears enables the detection of subtle morphological and deformability changes in RBCs related to storage lesions. This approach leverages widely available smear samples, suggesting a potentially accessible method for blood quality assessment in clinical settings(Lamoureux et al., 2024).
Automated Complete Blood Count and Blood Cell Recognition	3/45 Papers 25	Advanced image processing combined with deep learning facilitates automated recognition and counting of multiple blood cell types in smears, improving efficiency and accuracy over manual methods. These approaches support routine laboratory workflows and may extend to blood quality evaluation(Kamal & Raj, 2024; Yang & Li, 2023; Parayil et al., 2024).

Chronological Review of Literature

In the past decade, the research on the predictive quality of blood packets via machine learning has experienced a significant breakthrough. Early studies were conducted on the fundamental imaging procedures and measurements of physical entities to specify the morphology and deformability of red blood cells (RBC) during storage. Slowly, machine learning and deep learning methods are being integrated with the current imaging and microfluidic technology to enable automated, label-free, and non-destructive measurement of RBC quality. In recent articles, the influence of donor variability on blood quality and storage, and how scalable, clinically ready platforms that combine biophysical profiling and omics information can be applied to optimize transfusion, are pointed out.

Year Range	Research Direction	Description

2013–2015	Foundational Imaging and Biophysical Characterization	Initial work employed quantitative phase imaging and optical interferometry to quantify RBC morphology changes and membrane deformability during storage. Studies established correlations between storage duration and cell stiffness or hemolysis, proposing non-invasive imaging assays as potential quality indicators. Photoacoustic and holographic methods were used to assess oxygen-carrying capacity and biochemical changes in stored blood.
2018–2019	Development of Label-free Imaging Flow Cytometry and Morphology Classification	Label-free imaging flow cytometry (IFC) was introduced for high-throughput RBC morphology assessment, enabling rapid and objective classification of storage-induced morphological changes. Deep learning models began to replace manual expert annotation for RBC morphology classification, improving accuracy and reproducibility. Non-invasive microscopy and oblique back-illumination microscopy were adapted for blood bag assessment without opening containers.
2020–2021	Integration of Deep Learning with Automated Morphological and Biophysical Profiling	Studies demonstrated deep learning frameworks to classify RBC morphologies associated with storage lesions, using brightfield images and weak supervision to capture continuous degradation progression. Microfluidic platforms combined with machine learning enabled deformability and shape analysis relevant to disease and stored blood quality. Research expanded into antioxidant nanoparticle therapies to reduce oxidative stress during storage and further refined automated RBC classification pipelines.
2022–2023	Advanced Machine Learning for Quantitative Prediction and Omics Integration	Machine learning models were applied to predict hemoglobin and iron content in blood units, as well as assess donor and storage factors influencing RBC quality. Multi-modal approaches combined Raman spectroscopy, imaging, and biophysical data with AI for label-free, rapid blood quality evaluation. Large-scale scoping reviews highlighted the growing role and challenges of AI and ML in transfusion medicine, emphasizing clinical workflow integration, transparency, and multi-site validation. Omics data and big data analytics emerged as promising tools for personalized transfusion strategies.
2024	Clinical-Ready, Multimodal AI-Driven Platforms and Biophysical Profiling	Recent efforts focus on developing robust, label-free deep learning models with high accuracy for RBC morphology and deformability classification across multiple cohorts and clinical sites. Innovations include smartphone-enabled optofluidic diagnostic devices, microfluidic deformability cytometry, and CNN-based biophysical profiling from thin- film blood smears. Emphasis is placed on automating assessment workflows, addressing donor variability, and integrating AI with omics and big data for precision transfusion medicine. Emerging methods also explore non-destructive ultrasound and photoacoustic techniques for real-time blood quality monitoring.

Agreement and Divergence Across Studies

The literature is largely convinced by the potential of machine learning and label-free imaging techniques to provide the objective and high-throughput evaluation of red blood cell (RBC) quality in stored blood units. It is agreed that these methods will be as accurate as or more accurate than expert manual morphological classification, and allow non-destructive and fast analysis. Nevertheless, there is a divergence in the best imaging modalities, the exact machine learning architecture to use, and the focus on morphological and biophysical markers to predict the quality of prediction. Also, there is a variation in the coverage of clinical integration viability and cross-setting model generalizability. Such inconsistencies indicate variability in the study design, scale, and intended clinical uses.

Comparison Criterion	Studies in Agreement	Studies in Divergence	Potential Explanations
Predictive Model Performance	Several studies demonstrate that machine learning models achieve classification accuracy comparable to or exceeding human experts for RBC morphological assessment (~76-98% accuracy) (Doan et al., 2020; Doan et al., 2018; Routt et al., 2023 "Automated Red Blood Cell Classification...", 2022). Weakly supervised models also outperform traditional expert-based scoring in correlating with biochemical quality metrics (Doan et al., 2024; Doan et al., 2024).	Variability in reported accuracy and model types —Some studies favor fully Supervised deep CNNs (Doan et al., 2020; Doan et al., 2018); others use ensemble learning (Routt et al., 2023) or weak supervision (Doan et al., 2024). Accuracy ranges from ~76% in highly variable datasets to near 98% with ensemble methods (Routt et al., 2023).	Differences arise from imaging modalities (brightfield, phase imaging), dataset sizes, annotation strategies (expert-labeled vs weak labels), and class definitions. Some works prioritize generalizability across sites, while others focus on maximizing accuracy on curated datasets.
Imaging Technique Throughput	Label-free imaging flow cytometry (IFC) methods enable high-throughput analysis of tens of thousands of cells per sample, vastly exceeding manual microscopic methods (Doan et al., 2020) (Pinto et al., 2024) (Pinto et al., 2024). Quantitative phase imaging and holographic methods enable rapid non-contact assessment (Jiang et al., 2022; Bhaduri et al., 2015).	Some studies focus on microfluidics-based or optofluidic deformability cytometry with lower throughput but richer biophysical data (Mao et al., 2024; Rizzuto et al., 2021; Liang et al., 2024). Others emphasize static smears or RGB imaging with slower throughput (Lamoureux et al., 2024; Lamoureux et al., 2024).	Methodological divergence reflects trade-offs between throughput, morphological vs biophysical insights, and instrument complexity. High-throughput IFC favors morphology classification, while microfluidic deformability assays yield functional data at lower volumes.
Morphological Classification Accuracy	Automated deep learning classifiers closely approximate expert-level accuracy in categorizing RBC morphologies linked to storage lesions, with inter-expert agreement of ~82.5% and model-expert agreement ~76-86% (Doan et al., 2020; Doan et al., 2018; Doan et al., 2024). Morphological indices derived from imaging correlate with biochemical hemolytic assays (Doan et al., 2020; Boecker et al., n.d.).	Some studies report challenges in classifying intermediate or subtle morphology classes, with continuous rather than discrete morphological changes complicating labeling (Doan et al., 2020; Pinto et al., 2019). Others include more morphological classes or rare pathological shapes, complicating classification (Routt et al., 2023; Parayil et al., 2024).	Variability in Morphological taxonomy and labeling protocols influence classification difficulty. Weakly supervised models attempt to overcome subjective labeling limitations. Differences in image resolution and staining also affect accuracy.

Comparison Criterion	Studies in Agreement	Studies in Divergence	Potential Explanations
Sensitivity to Donor and Storage Variables	Multiple studies confirm donor-specific variability significantly affects RBC quality and morphology during storage, detectable via ML models and imaging (Işıkşacan et al., 2023; Pinto et al., 2024; Lamoureux et al., 2024; Pinto et al., 2024). Storage duration correlates with morphological degradation and biochemical hemolysis (Doan et al., 2020; Doan et al., 2024; Marin et al., 2022).	Some approaches focus mainly on storage time as a proxy for quality, neglecting donor heterogeneity, while others integrate donor metadata or omics to predict variability (Epah et al., 2022; D'Alessandro, 2023; D'Alessandro, 2024). The degree to which models generalize across donors and sites varies (Doan et al., 2024; Aynard et al., 2023).	Differences relate to data availability and study focus. Studies with large, multi-site datasets better capture donor effects. Omics integration is emerging but not yet standard. Weak supervision models improve robustness to variability.
Clinical Integration Feasibility	Label-free, automated imaging combined with ML shows potential for integration into blood bank workflows by reducing laborious manual assessment and sample handling (Doan et al., 2020; Doann et al., 2018; Yang et al., 2023). Web-based tools for hemoglobin/iron content prediction facilitate clinical adoption (Epah et al., 2022).	Challenges remain, including cost and complexity of imaging systems, need for extensive training data across facilities, and adaptation to resource-poor settings (Doan et al., 2020; Maynard et al., 2023; Lopes et al., 2023). Some studies propose microfluidic or low-cost devices but face throughput or operational limitations (Mao et al., 2024; Rizzuto et al., 2021).	Feasibility depends on balancing accuracy, speed, cost, and ease of use. Imaging flow Cytometers and high-end microscopes impose cost barriers. Microfluidic devices offer portability but may lack throughput. User training and data standardization also impact integration.

Theoretical and Practical Implications

According to theoretical and practical implications, this has been achieved through the introduction of machine learning with label-free imaging, such as imaging flow cytometry, quantitative phase imaging, where the historical characterization of red blood cell (RBC) storage lesions is no longer dependent on subjective professional assessments and where morphological progressions are made chronologically more conservative to physiological quality indicators (Doan et al., 2020; Doan et al., 2018; Doan et al., 2024). The fact that weakly supervised models of learning are used breaks the existing paradigms. They are based on the time of storage, but not the human annotation, meaning that they may help to reveal the natural degradation patterns of RBCs. They may also suggest that machine learning can be used to reveal hidden processes in biodata that cannot be seen by the human eye (Doan et al., 2020; Doan et al., 2024). The mentioned heterogeneity of RBC quality in terms of donor-specific factors (age, sex, genetics) and storage conditions may be used to endorse the new theory that posits that blood units are biologically customized products that have to be treated individually as part of transfusion medicine driven by the fusion of omics and machine learning (Işıkşacan et al., 2023; D'Alessandro, 2023; D'Alessandro, 2024). To fill the gap between blood cell biomechanics and transfusion efficacy, innovations in machine learning and optofluidic and microfluidic technology have been suggested to evaluate RBC deformability and elasticity as biophysical indices of blood quality as new theoretical frameworks (Mao et al., 2024, "Assessing red blood cell deformability f...", 2022; Bachraty et al., 2024). The Theorization of delicate morphological and deformability variations that prove to be difficult to examine in humans due to the application of deep learning to the high-throughput morphological classification and biophysical profiling of the RBCs using thin-film smears and microfluidic apparatuses suggests that the Biomarkers can be discovered by the use of AI (Lamoureux et al., 2024; Lamoureux et al., 2024; Rizzuto et al., 2021). The term big data, omics, and

AI in transfusion medicine indicates that precision transfusion approaches are currently shifting beyond a more realistic and complex interaction between donor biology, storage lesions, and recipient outcomes beyond simplistic chronological measures of storage (D'Alessandro, 2023; D'Alessandro, 2024; D'Alessandro and Bugert, 2023). Practically, machine learning-based lab-free imaging systems offer real-life, non-destructive, and fast-studying instruments that will have the potential to be utilized instead of the work-intensive and destructive traditional blood bank tests and will improve workflow and standardization of RBC quality examination (Doan et al., 2020; Pinto et al., 2024; Pinto et al., 2019). The accuracy of machine learning predictive models of hemoglobin and iron levels in packed RBC units allows avoiding iron overload in chronically transfused patients because of proper dosage and after (Epah et al., 2022). The introduction of computerized systems of morphological classification of the RBC based on deep learning and microfluidic technologies can assist in managing the patient individually and track the progress of the therapy (Rizzuto et al., 2021), as well as substantially enhance the quality of hereditary hemolytic anemias and other blood disorders diagnosis (Yang and Li, 2023). A potential solution to monitor and control the quality of blood products in real time by non-invasively analyzing the deformability of red blood cells and biophysical characteristics, and to develop safer and more effective blood products, is image-based and optofluidic cytometry based on AI (Assessing red blood cell deformability f..., 2022; Cien et al., n.d.). Despite these results, clinical translation must account for heterogeneous donor populations and the subject of different storage environments and be integrated into existing blood bank systems, comprising collaboration of clinicians, engineers, and data scientists (Doan et al., 2024; Maynard et al., 2023). Furthermore, the ever-growing information on donor-specific variations in RBC storage quality may influence policy development concerning donor selection and blood products in the process of ensuring that the machine learning-based information on omics and biomarkers is used to develop individualized transfusion regimes and reduce waste (İşiksacan et al., 2023; D'Alessandro, 2023; Pinto et al., 2024).

Limitations of the Literature

Most of the studies use small samples or small amounts of blood, and this hinders the generalization and external validity of such findings, thereby making it difficult to draw concrete conclusions about the variety of samples of the donor population and manufacturing procedures (Doan et al., 2018; Doan et al., 2024; Lamoureux et al., 2024; Dybas et al., 2022). The problem is that the studies are usually being performed in a single manufacturing facility or center, and the process of their preparation and the donor population are limited to one or several clinical facilities, which cannot be generalized to other clinical facilities and blood bank activities (Doan et al., 2018) (Doan et al., 2024; Pinto et al., 2024). Most machine learning models and new assessment procedures are not validated to demonstrate their predictive capabilities and use them in practice, which most of the time are not convincing enough to be used in practice (Doan et al., 2024; Maynard et al., 2023; Lopes et al., 2023). Subjectivity and inter-observer variability. Due to the expert visual classification applied in the course of training and validation, which introduces subjectivity and limits the reproducibility, the expert visual classification will not be objective and standardized (Doan et al., 2020; Doan et al., 2024; Pinto et al., 2019). Others feature the study of a limited number of morphological classes of RBCs, which disregards the entire range of morphological variations due to storage, and may hurt the sensitivity and specificity of classification models to detect small or infrequent morphological variations (Jiang et al., 2022; Liang et al., 2024; "Automated Red Blood Cell Classification... 2022). It is also believed that many studies fail to consider the donor-specific variables (e.g., age, sex, genetics) in the predictor models, which restricts the comprehension of their role in the quality of RBCs and transfusion outcomes and, consequently, the quality of models (İşiksacan et al., 2023; Epah et al., 2022; D'Alessandro, 2024; D'Alessandro and Bugert, 2023). Reproducibility and external validation of machine learning models are also threatened by the presence of publicly available datasets and source code that limit the creation of scientific research and translation into clinical applications (Maynard et al., 2023). More sophisticated devices and imaging and microfluidics are confined, prohibitively expensive (equipment and competencies), and are not readily obtained and scaled within standard blood bank protocols, particularly in smaller resource centers (Doan et al., 2020; Doan et al., 2024) (Chen et al., n.d.). Certain machine learning models can be optimised to particular datasets or imaging conditions and thus cannot extrapolate to new populations, instruments, or clinical sites as well, constraining the extrapolation (Doan et al., 2018; Doan et al., 2024; Maynard et al., 2023). Lastly, limited research has linked the measurements of RBC quality or machine learning measurements with patient transfusion outcomes, which are both required to determine the clinical relevance and directions of transfusion measures (Maynard et al., 2023; Lopes et al., 2023).

Gaps and Future Research Directions

The research priorities (High, Medium, Low) in this table reflect the potential impact of each identified research gap on clinical applicability, patient safety, predictive performance, model reliability, and the

overall advancement of machine learning-based blood quality assessment. High-priority gaps are significant gaps that have a direct impact on clinical translation and clinical decision-making that need to be addressed immediately. Medium-priority gaps represent significant gaps that can have a noticeable impact on system performance, but are not as pressing. Low-priority gaps are those that provide an opportunity for further improvements and can be considered when more important gaps have been rectified.

Gap Area	Description	Future Research Directions	Justification	Research Priority
Limited donor diversity and sample size in ML models	Many ML models for RBC quality prediction are trained on limited datasets from single sites with small donor pools, restricting generalizability across populations and blood bank settings.	Conduct large-scale, multi-center studies incorporating diverse donor demographics, preparation procedures, and manufacturing facilities to train and validate ML models.	Broader datasets are essential to capture biological variability and improve the model robustness and clinical applicability (Doan et al., 2018; Doan et al., 2024)(Pinto et al., 2024).	High
Lack of prospective clinical validation of ML-based assessments	Few studies have prospectively validated ML models or integrated them into routine clinical blood bank workflows, limiting evidence of real-world utility.	Design and implement prospective clinical trials and multi-site validation studies to assess ML model performance in operational blood bank environments.	Prospective validation is critical to demonstrate clinical effectiveness, reproducibility, and regulatory acceptance (Doan et al., 2018; Ynard et al., 2023; n et al., 2024).	High
Integration of biochemical and morphological markers	Current ML models often focus on morphology or biochemical markers separately, missing a comprehensive assessment of RBC quality combining both data types.	Develop multimodal ML frameworks integrating label-free imaging, biochemical assays, and omics data to improve the prediction of storage lesions and transfusion outcomes.	Combining Complementary data sources can enhance sensitivity and specificity beyond morphology or biochemistry alone (Doan et al., 2020; Dybas et al., 2022; D'Alessandro & Bugert, 2023).	High
Sensitivity to subtle biochemical changes undetectable by imaging	Label-free imaging methods may fail to detect early or molecular-level storage lesions that do not manifest as morphological changes.	Incorporate advanced spectroscopic techniques (e.g., Raman, diffuse reflectance) with ML to detect biochemical alterations non-invasively in stored samples. RBCs.	Early detection of biochemical changes is crucial for timely quality assessment and preventing transfusion complications (Dybas et al., 2022; Can & Ülgen, 2018; Hou et al., 2020).	Medium

Gap Area	Description	Future Research Directions	Justification	Research Priority
Standardization and Reproducibility Challenges	Heterogeneous Methodologies, lack of standardized protocols, and limited data/code sharing hinder reproducibility and benchmarking of ML models.	Establish standardized data collection, annotation, and reporting frameworks; promote open-source sharing of datasets and ML code for external validation.	Standardization facilitates comparison across studies and accelerates clinical translation (Maynard et al., 2023; Routt et al., 2023).	High
Limited functional biophysical assessment integration	Functional assays of RBC deformability and elasticity are often separate from morphological ML models and require specialized equipment, limiting clinical throughput.	Integrate biophysical deformability measurements with imaging-based ML models using accessible platforms (e.g., microfluidics, optofluidics) to enable high-throughput functional quality assessment.	Functional properties are key predictors of transfusion efficacy and should complement morphology-based assessments (Lamoureux et al., 2024; Mao et al., 2024 "Assessing red blood cell deformability f...", 2022; Rizzuto et al., 2021).	Medium
Challenges in classifying transitional RBC morphologies	ML models face difficulty distinguishing RBCs in transitional or adjacent morphological states due to the biological continuum and image variability.	Develop hierarchical or ensemble ML architectures with uncertainty quantification to better classify borderline morphologies and improve interpretability.	Accurate classification of subtle morphological changes is essential for precise staging of storage lesions (Doan et al., 2020; Routt et al., 2023; Parayil et al., 2024).	Medium
Limited exploration of donor-specific and environmental factors	Donor biology, genetics, and environmental e-Exposures significantly affect RBC storage quality but are underrepresented in predictive models.	Incorporate donor metadata, genetic, and exposome data into ML models to personalize blood quality predictions and optimize transfusion strategies.	Personalized models can improve transfusion safety and efficacy by accounting for inter-donor variability (İşıksaça et al., 2023; D'Alessandro, 2023; D'Alessandro, 2024).	High

Cost and complexity barriers for clinical adoption	Specialized imaging and microfluidic equipment, as well as computational demands, limit the widespread adoption of ML-based RBC quality assessment in blood banks.	Develop low-cost, portable, and user-friendly devices (e.g., smartphone-based optofluidic systems) integrated with ML for point-of-care blood quality monitoring.	Affordable and accessible technologies are needed to facilitate routine clinical implementation (Chen et al., n.d.; Doan et al., 2018; Yang et al., 2023).	Medium
Insufficient correlation with clinical transfusion outcomes	Most studies focus on surrogate markers of RBC quality without a robust linkage to patient transfusion outcomes or efficacy	Conduct longitudinal studies correlating ML-predicted RBC quality metrics with clinical outcomes such as post-transfusion recovery and adverse events.	Validating predictive models against clinical endpoints is necessary to establish clinical relevance (Doan et al., 2024; Maynard et al., 2023).	High

Overall Synthesis and Conclusion

All the literature reviews mentioned on the predictive quality measurement of blood packets with the assistance of machine learning before transfusion all emphasize massive advances in non-invasive analysis of red blood cells (RBCs) stored through automated techniques. Other machine learning models, such as deep learning architectures, are highly precise in classifying RBC morphological subtypes as well as predicting storage lesions and have often been found to be as accurate or more so than human analysis. The benefits of such models are that, since large-scale, label-free imaging technologies, such as imaging flow cytometry, quantitative phase imaging, and digital holographic microscopy, enable the analysis to be high-throughput and objective, and that images can be acquired without the use of any cell-damaging technology. Further predictive robustness to human labeling bias and further overlabeling of weakly supervised learning strategies, in which storage duration is taken as a proxy label, has also been achieved. Machine learning imaging modalities are a comprehensive phenotypic description of RBCs, which encompasses morphological degradation and biophysical values of deformability and elasticity. Such approaches are sensitive to the extent that they can be employed to trace small morphological variations with respect to donor variability, storage period, and biochemical variations. Integration of omics data suggests that genetic, environmental, and processing factors play an important role in the variability of the quality of the stored blood. This multidimensional knowledge elevates to a greater level the opportunities of personalized transfusion techniques in relation to the profile of the donor and that of the recipient. The same has been observed to pose a challenge even with these developments in transferring these technologies to the daily clinical blood banking processes. It has limitations of having larger heterogeneous datasets to ensure that it can be extrapolated to other populations and other manufacturing sites, not to mention that prospective multicenter validation is a precondition. The success of user-friendly instruments like the web application and portable optofluidic devices enhances the possibility of integrating the clinical systems and making it easy and easy to conduct a real-time assessment. However, imaging protocol and model reporting standardization and remedies to operational, regulatory, and cost barriers are imperative to wide adoption. The combination of label-free high-throughput imaging, machine learning, and biophysical profiling provides, in general, a disruptive solution to the issue of blood quality analysis. In spite of the fact that the analysis of predictive models is credible in order to characterize the change of RBC morphology and deformability in relation to storage lesion, full characterization of the variations related to donors and storage is an objective that is under development. To fulfill the goal of automated, objective, and personalized blood quality determination that enhances the safety and the effectiveness of transfusion, the constant process of method standardization, their further clinical validation, and the multimodal incorporation of the data will be required.

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