Review Article

A Review of IRF and RMI in Evaluating Regenerative and Non-regenerative Anemia in Dogs and Cats

Mahmood Ahmadi-hamedani[1*], Zeinab Bagherian[2]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
2. Student Research Committee, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.

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ABSTRACT

Anemia is one of the most common hematological topics in dogs and cats, and it has various causes and outcomes. The immature reticulocyte fraction (IRF) and reticulocyte maturity index (RMI) serve as emerging indicators that identify highly immature reticulocytes containing large amounts of ribonucleic acid (RNA). These parameters are critical for the immediate evaluation of bone marrow response, the precise differential diagnosis of various causes of anemia, the examination of treatment efficacy, and the control of bone marrow regeneration in different healthcare settings. The maturity of reticulocytes and the RNA fluorescence intensity are identified by systems such as Sysmex XN-1000V and ADVIA 2120i. These hematology analyzers precisely calculate these indices. The IRF and RMI in dog and cat species with hemolytic anemias are significantly higher than those in hemorrhagic anemia. Additionally, these reticulocyte parameters in dogs and cats with bone marrow failure (BMF) are considerably lower than in other non-regenerative anemia, and both groups compared to pre-regenerative anemia. This review article addresses the concepts, clinical applications, and comparison of the IRF and RMI determined by the Sysmex XN-1000V and ADVIA 2120i hematology analyzers in various types of regenerative, non-regenerative, and pre-regenerative anemia in dogs and cats.

Keywords: Blood cell regeneration, Bone marrow response, Diagnostic indicators, RNA fluorescence, Veterinary medicine

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*** Corresponding Author:**
Mahmood Ahmadi-hamedani, Associate Professor.
Address: *Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.*
Phone: *+98 (23) 31532603*
E-mail: *Ahmadi.hamedani@semnan.ac.ir*

Introduction

Anemia is one of the most common laboratory abnormalities in small animals (Comazzi et al., 2004). The differential diagnosis of anemia typically requires taking the patient history, examining clinical signs, assessing biochemistry, and serial evaluation of several hematological parameters (Perez-Ecija et al., 2024).

Anemia is generally classified into two categories: Regenerative (RA) and non-regenerative (NRA), based on the bone marrow's response, which is evaluated through the absolute reticulocyte count (ARC) (Tvedten, 2022). RA and NRA can be further categorized based on the underlying mechanisms of anemia (e.g. hemorrhage (HEM) or hemolysis in RA; bone marrow failure (BMF) or other causes in NRA). Red blood cell and reticulocyte indices are warning signs and valuable in diagnosing and managing anemia (Jung et al., 2023).

In 1865, Wilhelm Heinrich described reticulocytes as granular, immature red blood cells containing a relative amount of RNA. They can show the anemia regeneration model and bone marrow response to the treatment (Piva et al., 2015; Wollmann et al., 2014). Automated counting of reticulocytes is now possible through methods that utilize fluorescence detection or variations in light absorption. The fluorochromes and dyes include polymethine, acridine orange, and thiazole orange based on the equipment used, including oxazine 750 and new methylene blue. As previously reported, reticulocyte analysis using flow cytometry presents an attractive alternative, offering higher reproducibility than microscopic counting methods (Van Houte et al., 1994).

Reticulocytes can be classified into three categories based on their maturity, measured by RNA content and the intensity of fluorescence or light absorption emitted. These classifications are as follows: Fraction I (high fluorescence reticulocyte; HFR), fraction II (medium fluorescence reticulocyte; MFR), and fraction III (low fluorescence reticulocyte; LFR) (Jung et al., 2023). Additionally, new reticulocyte indices have been introduced based on automation features. These include the mean reticulocyte volume (MCVr) for volume measurement and reticulocyte hemoglobin content (CHr) for hemoglobin assessment, along with measures for volume dispersion (Moloney et al., 2023).

Iron deficiency (ID) usually goes through specific biochemical markers, such as serum ferritin level, percentage transferrin saturation, soluble transferrin receptors, and serum hepcidin (Ganz & Nemeth, 2012; Ahmadi-hamedani, 2019). These biomarkers are significantly affected by diurnal variation, inflammation, infection, and malignancy. However, these tests may be costly, and not all health centers can afford to carry out them. Reticulocytes remain in the bloodstream for four days before fully mature into erythrocytes. Meanwhile, erythrocytes stay in circulation for approximately 120 days in humans and dogs and 70 days in cats. Given this information, changes in the volume and content of reticulocytes occur much more rapidly than those of erythrocytes. This characteristic of reticulocytes makes them suitable for differential diagnosis and assessment of the response to anemia treatment by calculating previously mentioned new indices (Kılıç et al., 2022; Keiner et al., 2020; Briggs, 2009).

Many investigations have shown that CHr can identify the presence of ID even when anemia is absent. A CHr level below the age-specific lower limit of the reference range indicates ID in infants (Ennis et al., 2018; Pomrop et al., 2020; Düzenli Kar & Altınkaynak, 2021). Reticulocyte indices, especially CHr, signal early iron-limited erythropoiesis (ILE) in dogs, but neither CHr nor iron metabolism variables can reliably distinguish the different underlying causes of ILE in dogs (Schaefer & Stokol, 2016; Ahmadi-hamedani, 2019).

The reticulocyte count (RET) is clinically significant for the pathophysiological classification of anemia. A lower value of RET means that the bone marrow is not producing enough red blood cells. Meanwhile, a high level of reticulocyte production taxes the cell production line to churn out more red blood cells that have been destroyed or due to massive bleeding (Cascio & DeLoughery, 2017). RET cannot distinguish the various causes of anemia. Furthermore, due to the delay in reticulocyte release into the bloodstream, pre-regenerative anemias (PRA; acute bleeding) may be mistakenly diagnosed as NRA (Jung et al., 2023).

The immature reticulocyte fraction (IRF) is a relatively new indicator in measuring reticulocytes. It refers to immature reticulocytes that contain significant amounts of RNA, including MFR and HFR, and it has more excellent reproducibility than HFR. This RNA can be accurately stained with certain fluorescent dyes for nucleic acids, such as polymethine and oxazine 750, and was automatically analyzed by flow cytometry (Sysmex XN-1000V and ADVIA 2120i) (Adane & Asrie, 2021). Stud-

ies have shown that an increase in IRF is superior to other hematologic indices, such as RET, as an early marker of bone marrow recovery or hematopoietic stem cell transplantation (Piva et al., 2015). The IRF is essential for several purposes in making a helpful clinical decision. It is used to assess bone marrow status post-chemotherapy (Raja-Sabudin et al., 2014), evaluate anemia diagnosis and management (Geldard et al., 2009), diagnose aplastic anemia (Sindhu et al., 2016), and determine when patients with anemia require an RBC transfusion (Raja-Sabudin et al., 2014).

In the study by Melendez-Lazo et al. (2015), it was demonstrated that diseased dogs with elevated levels of C-reactive protein exhibited a significantly higher reticulocyte red cell distribution width, a more significant percentage of hypochromic reticulocytes, a higher percentage of reticulocytes with low cellular CHr, and a lower IRF compared to healthy dogs. The Dogues de Bordeaux breed exhibits lower IRF and MFR percentages than other dog breeds, while their LFR and HFR percentages are high (Lavoue et al., 2014). No significant statistical difference was observed in the reticulocyte indices (IRF, LFR, MFR, and HFR) between dog blood samples collected in tubes containing EDTA (ethylenediaminetetraacetic acid) and those containing citrate-theophylline-adenosine-dipyridamole (CTAD). Furthermore, the reticulocyte indices demonstrate a strong correlation between the two types of tubes, EDTA and CTAD (Granat et al., 2017). It has been shown that reticulocyte indices (LFR, MFR, HFR, and IRF) in feline blood samples, measured with Sysmex XT-2000iV in EDTA or EDTA-CTAD tubes, remain stable after 48 hours of storage at room temperature (Granat et al., 2013). Reticulocyte indices (RET, LFR, MFR, and HFR) in feline blood show consistent stability with both EDTA and EDTA-CTAD anticoagulants when measured using Sysmex XT-2000iV and ProCyte DX, with only minor variations between analyzers.

Guerlin et al. (2024) validated the Sysmex XN-V analyzer for canine blood, confirming the accuracy of RET, LFR, MFR, and HFR with EDTA, compared to the XT-2000iV, though delays caused some variations.

Reticulocyte maturity index (RMI) is expressed in percentage form and can be determined by the Equation 1:

1. $RMI=[(MFR+HFR)\times100]/LFR$

This calculation is based on ratios of various sub-populations in reticulocytes and is one of the best indicators for erythropoiesis (Urrechaga et al., 2011). In human medicine, as mentioned above, IRF is widely used for the early diagnosis of erythropoietic response following bone marrow transplantation or chemotherapy and for classifying types of anemia based on regeneration status (Piva et al., 2015; Raja-Sabudin et al., 2014).

Only four studies have directly examined the reference intervals of IRF and RMI in veterinary medicine and its application in diagnosing different types of anemia in small animals. One study conducted in 2023 reported the IRF reference intervals in 106 adult large-breed dogs using the ADVIA 2120 hematology analyzer (Moloney et al., 2023). Another study evaluated the use of the IRF to identify early red blood cell regeneration in 45 anemic dogs, utilizing the ADVIA 2120i analyzer in 2023 (Jung et al., 2023). The third study, published in 2024, is more comprehensive than the previous two. It assessed the IRF concerning the causes of regenerative and non-regenerative anemia in dogs and cats using the Sysmex XN-1000V hematology analyzer (Perez-Ecija et al., 2024). The last article discusses the diagnostic application of the RMI obtained from the Sysmex XN V analyzer in various types of anemia in dogs, as presented by Perez-Ecija et al. (2024).

To the best of the authors' knowledge, no detailed study has been conducted on using IRF and RMI to diagnose different types of anemia in dogs and cats. Therefore, the present study aimed to provide an overview of the clinical application of IRF and RMI in the differential diagnosis of various types of anemia in dogs and cats.

Materials and Methods

Review protocol development and search strategy

The protocol for this review was developed following the essential reporting items outlined in the PRISMA (the preferred reporting items for systematic reviews and meta-analyses) checklist (Moher et al., 2009). An extensive literature review was conducted on the potential of the IRF and RMI for evaluating regenerative, non-regenerative, and pre-regenerative anemia in dogs and cats in PubMed, ScienceDirect, and Google Scholar, using the key terms ((“immature reticulocyte fraction” OR “reticulocyte maturity index”) AND (“anemia”) AND (“veterinary”)) between 2000-2024. Figure 1 shows the PRISMA flow diagram for document selection. We finally found four articles with appropriate information for this review article. Only English-language studies published until December 29, 2024, were considered. No relevant systematic reviews on this topic were found. The research articles eligible for review described the

clinical characteristics of patients with regenerative, non-regenerative, and pre-regenerative anemia and utilized IRF and RMI as diagnostic parameters. Our review included prospective and retrospective cohort, case-control, and cross-sectional studies.

Study selection

We reviewed the initial search results, focusing on the titles and abstracts. Next, we closely examined the full texts of the articles that appeared to be relevant to determine if they met our inclusion and exclusion criteria. We eliminated duplicate articles, case reports, expert opinions, letters, editorial pieces, and human studies. Additionally, we excluded studies that did not include control groups or did not involve dogs and cats. We also discarded any studies without available data, articles in languages other than English, and those that focused exclusively on IRF and RMI.

Data extraction

The information collected includes article details (first author, publication year), species, device specifics, the parameters investigated, and key findings presented in Table 1. A Microsoft Excel database was utilized to store the relevant information.

Discussion

Evaluation of IRF and RMI in various types of regenerative anemia

Perez-Ecija et al. (2024) established that in regenerative anemia, the mean value of IRF estimated by Sysmex XN-1000V hematology analyzer in dogs and cats was significantly higher than that in healthy animals, 47.2% and 57.9% compared to 22.7% and 35.6%, respectively. Furthermore, the mean value of this fraction in regenerative anemia resulting from hemolysis in both dog and cat species was significantly higher (54.2% and 69.9%, respectively) compared to anemia due to HEM (37% and 41.1%). In animals with regenerative anemia, the IRF (%) demonstrated excellent accuracy in cats (cut-off: 54.3, sensitivity: 96.4%, specificity: 80%, and area under curve: 0.936) and dogs (cut-off: 44.7, sensitivity: 87.2%, specificity: 71.3%, and area under curve: 0.834) for distinguishing between HEM and hemolysis (LYS) anemia (Table 2). The median value of IRF reported by Jung et al. (2023) using the ADVIA-2120i hematology analyzer in dogs with regenerative anemia (46.5%) was significantly greater than that in healthy dogs (22.1%). Based on the studies by Perez-Ecija et al. (2024) and Jung et al. (2023), IRF in regenerative anemia in dogs and cats is significantly higher than that in healthy animals, and this increase is more pronounced in LYS anemia compared to HEM anemia in both species. Utilizing the numerical data regarding low, medium, and high fluorescence reticulocytes reported in the study by Perez-Ecija et al. (2024), the authors of the present study calculated the

Table 1. Overview of studies on reticulocyte indices (LFR, MFR, HFR, IRF, and RMI) in dogs and cats with various types of anemia

Authors, Year	Species (Sample Size)	Hematology Analyzer and Parameters	Key Findings
Perez-Ecija et al. (2024)	Dogs (healthy=48; Hemolysis=50; Acute HEM=603; PRA=25; Bone marrow aplasia=15; Bone marrow damage=15)	Sysmex XN-V LFR, MFR, HFR, IRF, and RMI	Reference ranges have been established for the different reticulocyte maturity fractions (LFR, MFR, HFR, IRF) determined with the Sysmex XN-V analyzer.
Jung et al. (2023)	Dogs (healthy=49; anemia=45)	ADVIA 2120i IRF	Measuring the IRF could assist in classifying anemic dogs more accurately.
Moloney et al. (2023)	Large-breed adult dogs (n=106)	ADVIA 2120 Reference intervals for reticulocyte indices, IRF, and HYPO-RBC	This study established reference intervals for reticulocyte indices and novel hematological variables in healthy blood donor dogs.
Perez-Ecija et al. (2024)	Dogs (healthy=2654; RA=223; NRA=603; PRA=137) Cats (healthy=393; RA=48; NRA=148; PRA=35)	Sysmex XN-1000V IRF	The assessment of the IRF in the Sysmex XN-1000V analyzer can assist veterinarians in more accurately classifying the type of anemia in dogs and cats.

Abbreviations: HFR: High reticulocyte fraction; HYPO-RBC: Hypochromic red blood cell; IRF: Immature reticulocyte fraction; LFR: Low reticulocyte fraction; MFR: Medium reticulocyte fraction; NRA: Non-regenerative anemia; PRA: Pre-regenerative anemia; RA: Regenerative anemia.

RMI in healthy dogs and cats, as well as in cases of various types of regenerative, pre-regenerative, and non-regenerative anemia. This index is derived from the ratio of the total medium and high fluorescence reticulocytes to low fluorescence reticulocytes. In this context, the RMI in dogs and cats with regenerative anemia (0.84 and 1.37, respectively) was higher than in healthy animals (0.28 and 0.55). Additionally, the RMI in LYS-induced regenerative anemia in dogs and cats (1.87 and 2.32, respectively) was significantly higher than in HEM-induced anemia (0.55 and 0.70). These findings align closely with the IRF results in distinguishing between hemolytic and hemorrhagic regenerative anemia in dogs and cats, highlighting the greater intensity and speed of compensatory response in hemolysis-induced anemia (Table 2).

Evaluation of IRF and RMI in various non-regenerative anemia

The mean IRF value shows significant differences among non-regenerative anemia in dogs and cats (25.4% and 41.4%, respectively) compared to pre-regenerative and regenerative anemias, as well as among different causes of non-regenerative anemias in the study by Perez-Ecija et al. (2024). In BMF, due to the loss of the ability to produce immature reticulocytes by the bone marrow, the mean value of this fraction (14.6%) was significantly low (Pomrop et al., 2020). In other non-regenerative anemia resulting from inflammatory diseases, chronic renal failure, endocrinopathy, etc., the mean IRF (26%) was higher than that of non-regenerative anemia due to BMF (14.6%) but still lower than that of pre-regenerative or regenerative anemia caused by HEM and LYS. These results were also observed in cats, where the mean IRF in non-regenerative anemia due to BMF (25.9%) was significantly lower than that of other non-regenerative anemia caused by inflammatory diseases, chronic renal failure, endocrinopathy, etc. (42.5%) and pre-regenerative anemia. In non-regenerative anemia, the IRF showed good accuracy in cats (cut-off: 27.7, sensitivity: 85.9%, specificity: 76.4%, and area under curve [AUC]: 0.836) and moderate accuracy in dogs (cut-off: 16.2, sensitiv-

Table 2. IRF (%) and RMI (%) for healthy and different anemia types in dogs and cats

Condition	IRF (%)	RMI (%)	Cut-off	Sensitivity (%)	Specificity(%)	AUC
Healthy dogs	22.7	0.28				
Healthy cats	33.3	0.55				
RA dogs	47.2	0.84				
HEM	37.0	0.55	44.7	87.2	71.3	0.834
LYS	54.2	1.87				
RA cats	57.9	1.37				
HEM	41.1	0.70	54.3	96.4	80	0.936
LYS	69.9	2.32				
PRA-dogs	29.1	0.39	21.9	46.7	77.3	0.626
PRA-cats	54.5	1.20	53	84.9	68.5	0.761
NRA dogs	25.4	0.32				
BMF	14.6	0.16	16.2	79.6	55.4	0.722
OTH	26.0	0.33				
NRA cats	41.4	0.72				
BMF	25.9	0.34	27.7	85.9	76.4	0.836
OTH	42.5	0.74				

Abbreviations: AUC: Area under the curve; BMF: Bone marrow failure; HEM: Hemorrhage; IRF: Immature reticulocyte fraction; LYS: Hemolysis; NRA: Non-regenerative anemia; OTH: Other non-regenerative anemias; PRA: Pre-regenerative anemia; RA: Regenerative anemia; RMI: Reticulocyte maturity index.

ity: 79.6%, specificity: 55.4%, and AUC: 0.722) for distinguishing BMF from other causes of NRA (Table 2) (Perez-Ecija et al., 2024). Jung et al. (2023) reported that in dogs with non-regenerative anemia, the median value of this fraction (18.7%) was notably lower than in dogs with pre-regenerative and regenerative anemia. The RMI in dogs and cats with non-regenerative anemia caused by BMF (0.16 and 0.34, respectively) was lower than the values observed in other causes of non-regenerative anemia, such as inflammatory diseases, chronic renal failure, endocrinopathies, and similar conditions (0.33 and 0.74), reflecting the results of the IRF. These findings highlight a direct correlation between the underlying condition and the intensity of erythropoietic compensatory response. Differentiating between regenerative and non-regenerative anemia is feasible using varying levels of these indices. Hence, high IRF and RMI values represent active regenerative activity instead of those BMF represented by lower scores (Moloney et al., 2023).

Evaluation of IRF and RMI in pre-regenerative anemia

IRF is a powerful tool for the early diagnosis of pre-regenerative anemia. Perez-Ecija et al. (2024) found that the mean level of this fraction in dogs and cats with pre-regenerative anemia (29.1% and 54.5%, respectively) was significantly higher than in those with non-regenerative anemia due to BMF. Also, IRF showed a significant increase in dogs with pre-regenerative anemia (40.6%) in the study by Jung et al. (2023) compared to those with non-regenerative anemias. The IRF demonstrated moderate ability in cats (cut-off: 53, sensitivity: 84.9%, specificity: 68.5%, and AUC: 0.761) and poor ability in dogs (cut-off: 21.9, sensitivity: 46.7%, specificity: 77.3%, and AUC: 0.626) to differentiate between pre-regenerative anemia and other non-regenerative anemia types (Table 2) (Perez-Ecija et al., 2024). According to the calculations of the present study, RMI in dogs and cats with pre-regenerative anemia (0.39 and 1.20, respectively) was higher than the values associated with various types of non-regenerative anemia. This increase occurs before the rise in reticulocytes in peripheral blood is observed. It suggests that the IRF can identify early bone marrow activity in response to ID sooner than other indicators (Perez-Ecija et al., 2024). This indicator has been increased in dogs and cats with pre-regenerative anemias compared to non-regenerative anemias, even in the early stages of the disease when reticulocytosis has not yet become apparent. This finding assists veterinarians in accurately distinguishing pre-regenerative anemias from other types of anemia that may be confused with them in the early stages (Jung et al., 2023).

Comparing IRF and RMI with routine and novel anemia indices in dogs and cats

IRF and RMI show significant advantages over routine and novel indices, such as ARC, CHr, MCVr, hypochromic red blood cell percentage (%HYPO-RBC), and CH-delta (the difference in CHr between reticulocytes and mature red blood cells) (Moloney et al., 2023). These indices can determine the activity of the bone marrow in regeneration. They can do this even in the pre-regenerative state of anemia, where reticulocytes have not yet begun to manifest themselves. In contrast, the ARC only increases after the appearance of mature reticulocytes. Therefore, the IRF and RMI provide more accurate early detection of these types of anemias (Perez-Ecija et al., 2024). Unlike the routine indices mentioned above, the IRF and RMI can distinguish between regenerative anemia (such as ID anemia and hemolytic anemia) and non-regenerative anemia (such as anemia caused by chronic disease). This feature assists veterinarians in arriving at accurate results in a shorter time (Pomrop et al., 2020). ROC curve analysis showed that this fraction has higher accuracy than the reticulocyte production index in differentiating between pre-regenerative anemia and those caused by ID (Jung et al., 2023).

The role of the IRF and RMI in improving clinical decision-making in anemia

IRF and RMI enable veterinarians to assess bone marrow regeneration without needing prolonged, quick, repeated CBC monitoring (Jung et al., 2023). Both pre-regenerative anemia and regenerative anemia have increased IRF index values that mean active bone marrow production, which often may necessitate supportive care. On the other hand, the low value of this index in non-regenerative anemia may be actual, as it suggests further management of bone marrow diseases or non-regenerative anemia (Jung et al., 2023). IRF and RMI enable anemia to be detected right from the early stage, before the beginning of reticulocytosis. These indices can assess patients' treatment responses to anemia (Moloney et al., 2023). The Écija diagram is a valuable tool designed to assist in diagnosing various types of anemia in dogs by evaluating RMI. It emphasizes five key factors: 1) high HFR (≥26%) and high RET (≥150000/µL) suggest hemolytic anemia, 2) normal to high HFR (≥21%) and a RET between 100000 and 150000/µL indicate acute HEM, 3) normal HFR (5%≤ HFR ≤21%) and RET 20000-150000/µL pointing to normal conditions or another type of anemia, 4) low HFR (≤5%) and low RET (≤20000/µL) that suggests bone marrow aplasia, 5) normal to high HFR (>5%) but low RET (≤20000/µL) that may indicate dyserythropoietic or bone marrow damage (Perez-Ecija et al., 2024).

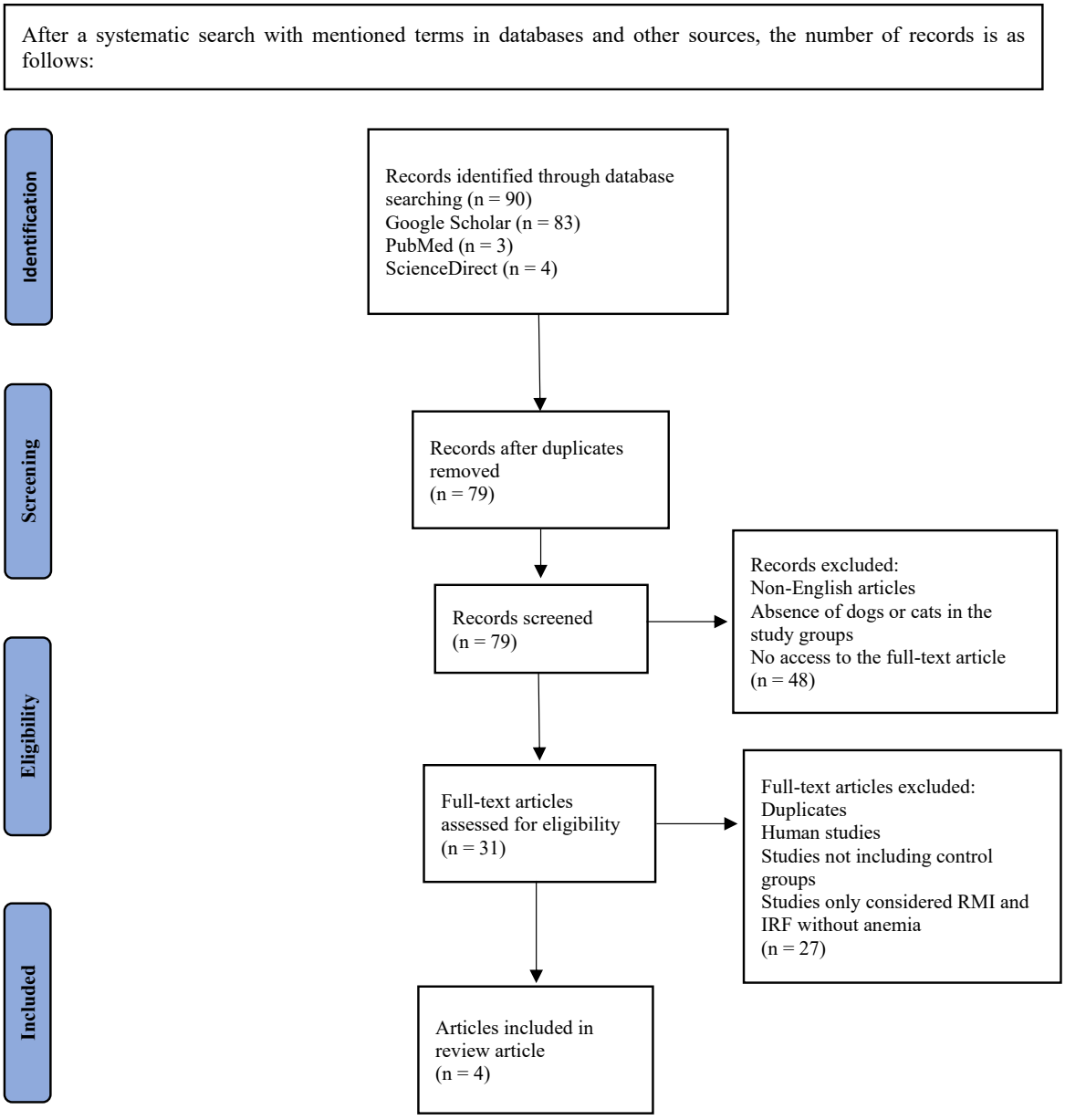

Figure 1. Flow diagram for inclusion of studies in the combined systematic review and meta-analysis (Moher et al., 2009)

Conclusion

According to the data presented in this review article, the values of IRF and RMI in dogs and cats with different types of regenerative anemia are higher than those in healthy individuals and those suffering from non-regenerative and pre-regenerative anemia, particularly in cats. Interestingly, the values of IRF and RMI in dogs and cats with regenerative anemia due to hemolysis also showed a significant tendency to increase compared to individuals with other types of anemia. IRF must be standardized against these global norms, requiring much work to ensure that the criteria used in different hematology analyzers are uniform. Some devices use the IRF index, others utilize the RMI, and some report only the percentage of IRF. Although all of these indices can reflect bone marrow activity, different methods for obtaining them can complicate the comparison of results. These indices' limitations include the need for valid external quality assessments and internal quality controls, which complicates result interpretation and clinical decision-making. However, new reticulocyte indices could significantly improve the diagnosis and monitoring of many blood disorders. Different hematology analyzers provide varying results for IRF, representing a significant weakness in the potential clinical applications of these indices in

dogs and cats. Mending this limitation will ensure the IRF index and other reticulocyte indices are crucial for the differential diagnosis of various types of anemia and for monitoring their treatment. Consequently, IRF and RMI are valuable for detecting anemia at its preliminary stage or discriminating one type from another in dogs and cats. When combined with other reticulocyte indices, IRF can increase diagnostic precision enormously and assist the veterinarian in achieving optimal care and treatment of anemia.

Limited studies regarding IRF in dogs and cats have been published since 2023. Owing to the general need for well-defined reference values for RMI in healthy individuals and those with different types of anemia, using these indices in everyday practice is difficult. Additionally, the reported reference values for IRF vary based on the kind of hematology analyzer used in the studies, and for this reason, the diagnostic threshold values are method-dependent. The results reported regarding the fraction of immature reticulocytes in the studies employed indicate high variability in the values of this parameter based on the hematology analyzer used. For example, in healthy dogs, the IRF values ranged from 22.7% (using the Sysmex XN-1000V device) to 26.9% (using the ADVIA-2120 device). Even among the ADVIA-2120 hematology analyzers, IRF values range from 22.1% (ADVIA-2120i) to 26.9% (ADVIA-2120). Similarly, hematology analyzers have reported significant differences in regenerative, non-regenerative, and pre-regenerative anemia types. Based on the significant differences in the results provided by various hematology analyzers, standardization of the indicators dependent on the analysis method remains necessary. IRF, RMI, and other novel reticulocyte indicators seem to be much more sensitive markers for sudden changes in erythropoiesis than conventional red blood cell indices.

Ethical Considerations

Compliance with ethical guidelines

This study is a narrative review with no involvement of human or animal samples.

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Authors' contributions

Conceptualization, methodology, data collection, data analysis, supervision, funding acquisition and resources: Mahmood Ahmadi-hamedani; Investigation, and writing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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