Ultrasound in Med. & Biol., Vol. 44, No. 7, pp. 1303–1317, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.02.020

● *Review*

QUANTITATIVE MEASUREMENT OF ERYTHROCYTE AGGREGATION AS A SYSTEMIC INFLAMMATORY MARKER BY ULTRASOUND IMAGING: A SYSTEMATIC REVIEW

Prajwal Gyawali,* Daniela Ziegler,† Jean-François Cailhier,‡,§ André Denault,¶,‖,# and Guy Cloutier*,**,††

* Laboratory of Biorheology and Medical Ultrasonics, University of Montreal Hospital Research Center (CRCHUM), Montréal, Québec, Canada; † Documentation Center, University of Montreal Hospital, Montréal, Québec, Canada; ‡ University of Montreal Hospital Research Center (CRCHUM), Montréal, Québec, Canada; § Department of Medicine, University of Montreal, Montréal, Québec, Canada; ¶ University of Montreal Hospital, Montreal, Québec, Canada; ‖ Montreal Heart Institute, Montreal, Québec, Canada; # Department of Anesthesiology, University of Montreal, Montréal, Québec, Canada; ** Department of Radiology, Radio-Oncology and Nuclear Medicine, Montréal, Québec, Canada; and †† Institute of Biomedical Engineering, University of Montreal, Montréal, Québec, Canada

(*Received* 17 *November* 2017; *revised* 21 *February* 2018; *in final form* 28 *February* 2018)

Abstract—This systematic review is aimed at answering two questions: (i) Is erythrocyte aggregation a useful biomarker in assessing systemic inflammation? (ii) Does quantitative ultrasound imaging provide the non-invasive option to measure erythrocyte aggregation in real time? The search was executed through bibliographic electronic databases CINAHL, EMB Review, EMBASE, MEDLINE, PubMed and the grey literature. The majority of studies correlated elevated erythrocyte aggregation with inflammatory blood markers for several pathologic states. Some studies used "erythrocyte aggregation" as an established marker of systemic inflammation. There were limited but promising articles regarding the use of quantitative ultrasound spectroscopy to monitor erythrocyte aggregation. Similarly, there were limited studies that used other ultrasound techniques to measure systemic inflammation. The quantitative measurement of erythrocyte aggregation has the potential to be a routine clinical marker of inflammation as it can reflect the cumulative inflammatory dynamics *in vivo*, is relatively simple to measure, is cost-effective and has a rapid turnaround time. Technologies like quantitative ultrasound spectroscopy that can measure erythrocyte aggregation non-invasively and in real time may offer the advantage of continuous monitoring of the inflammation state and, thus, may help in rapid decision making in a critical care setup. (E-mail: guy.cloutier@umontreal.ca)

Key Words: **Quantitative ultrasound imaging, Ultrasound spectroscopy, Critical care medicine, Point-of-care monitoring system, Erythrocyte aggregation, Inflammation, Backscatter coefficient, Structure factor.**

INTRODUCTION

Systemic inflammation is a condition associated with several metabolic disorders (Hotamisligil 2006), such as obesity, diabetes, cardiovascular diseases, pre-eclampsia (Schiessl 2007), rheumatoid arthritis (Choy and Panayi 2001), Alzheimer disease (Akiyama et al. 2000), Parkinson disease (McGeer and McGeer 2004), cancers (Mantovani 2005), chronic obstructive pulmonary diseases (Yamamoto et al. 1997) and other critical states, for example, traumatic brain injury (Ramlackhansingh et al. 2011), multiple organ failure (Goris et al. 1985) and cardiac surgery (Asimakopoulos 2001). The systemic inflammatory response syndrome (SIRS), with or without infection, is common in critically ill patients (Gustot 2011). The inflammatory cascade generated after a trauma has been considered a pathophysiologic basis of SIRS. Systemic inflammation is associated with physiologic deterioration and organ dysfunction in such patients (Muckart and Bhagwanjee 1997). It may affect multiple organs (in 10%–15% of cases) leading to poor patient outcomes and increased mortality, particularly in cases of intense vasoplegia (Maharaj and Laffey 2004). A mortality rate

Address correspondence to: Guy Cloutier, Laboratory of Biorheology and Medical Ultrasonics, University of Montreal Hospital Research Center, 900 Saint-Denis, Montreal, QC H2X 0A9, Canada. E-mail: guy.cloutier@umontreal.ca

of 41% was reported in the 10% to 15% of patients experiencing SIRS and multiple organ dysfunction (Warltier et al. 2002). However, the contribution of the inflammatory response to adverse patient outcomes is potentially reversible. Thus, the continuous quantitative measurement of inflammatory markers may offer an advantage for the management of critically ill patients (Urbach et al. 2004).

The continuous monitoring of inflammatory markers in the critical care setup, nonetheless, comes with its own challenges. More than 80 blood markers of inflammation (cytokines and chemokines, immune-related effectors, reactive oxygen and nitrogen species, acute phase proteins, prostaglandins and cyclooxygenase-related factors, mediators such as transcription factors and growth factors and procalcitonin) have been identified in the scientific literature (Brenner et al. 2014; Zakynthinos and Pappa 2009). The information provided by these biomarkers does not necessarily overlap (Ikonomidis et al. 2008), and thus, a single measurement of an inflammatory marker does not reflect the overall dynamics of the inflammation process *in vivo* (Leng et al. 2008; Libby et al. 2002). Moreover, comparisons of cytokine levels are often problematic for the clinician owing to the use of several different techniques to derive them (Leng et al. 2008). Levels of cytokines measured also depend on a number of preanalytical factors such as the blood sample processing and storage, feeding cycle of the patient, anticoagulants used and circadian patterns (Thavasu et al. 1992; Zhou et al. 2010), further complicating the interpretation process.

Undoubtedly, the measurement of several markers over a period through state-of-the-art technologies would provide a better picture of the inflammatory process. Intracellular staining of cytokines utilising fluorescence-activated flow cytometry (Freer and Rindi 2013), multiplex arrays based on flow cytometry, chemiluminescence or electrochemiluminescence have all been used as advanced methods, but these approaches require costly initial setup and highly trained staff (Leng et al. 2008). Rather than the cost and availability of the technology, the main concern, however, in the context of critical care, would be the turnaround time, which ranges from hours to days even with advanced techniques (Leng et al. 2008). These biochemical markers are then of no value when frequent monitoring and rapid medical decisions are required in the intensive care unit. Therefore, despite the valuable clinical information that can be obtained from many inflammatory markers, they have not been used effectively in critical care because of the unavailability of rapid and reliable tests that can be serially determined and could report the overall status of systemic inflammation generated *in vivo*.

We are thus in dire need of such a biomarker that has the potential to report the cumulative quantification of these inflammatory molecules reliably, is relatively simple to measure, is cost effective and has a rapid turnaround time. The quantitative measurement of erythrocyte aggregation could be the one valuable test to monitor the generalized inflammatory process detectable in the blood. In this context, the present systematic review aimed to find out if erythrocyte aggregation can be used as a marker to measure systemic inflammation. Moreover, our review also aimed to determine if non-invasive techniques such as ultrasound can be used to quantitatively measure erythrocyte aggregation *in vivo* in real time, so that it could be used in a critical care setup to serially assess systemic inflammation.

METHODS

The search was executed by an academic librarian (D.Z.) through bibliographic electronic databases CINAHL (from 1937 onwards), EMB Review (from 1991 onwards), EMBASE (from 1974 onwards), MEDLINE (from 1946 onwards), PubMed and the grey literature (CADTH, Clinical Trials, National Guideline Clearing House, National Institute for Health and Care Excellence [NICE], MedNar, Google Scholar and Open Grey). The search combined words and expressions for two conceptual groups: *erythrocyte aggregation* and *inflammation*. To obtain the ultrasonography aspect, we added terms and expressions combined with OR in the second conceptual group (inflammation). We used words and expressions from controlled vocabulary (MeSH, EMTREE and others) and free-text searching. Exact key words used for search in each database are given in Appendix 1. Retained articles had received institutional animal or human ethical committee approvals.

RESULTS

Results of literature search

The initial search through CINAHL, EMB Review, EMBASE, MEDLINE, PubMed and the grey literature identified 1996 references after removing duplicates. Of 1996, 298 were not considered because they were not written in English. One thousand four hundred three articles were not specific to our objectives and thus were excluded after reading the abstract. Further, 98 papers were not retained after careful reading of the full text because of the following reasons: (i) they described erythrocyte aggregation in the context of technical issues in measurements, nitric oxide metabolism, redox balance, anemia, use of biomedical devices and hemostatic agents, presence in several pathologic states, and storage and blood banking, without taking into account inflammation or inflammatory markers directly; (2) they described ultrasound in the context of blood coagulation and blood echogenicity without considering erythrocyte aggregation; and (3) they studied

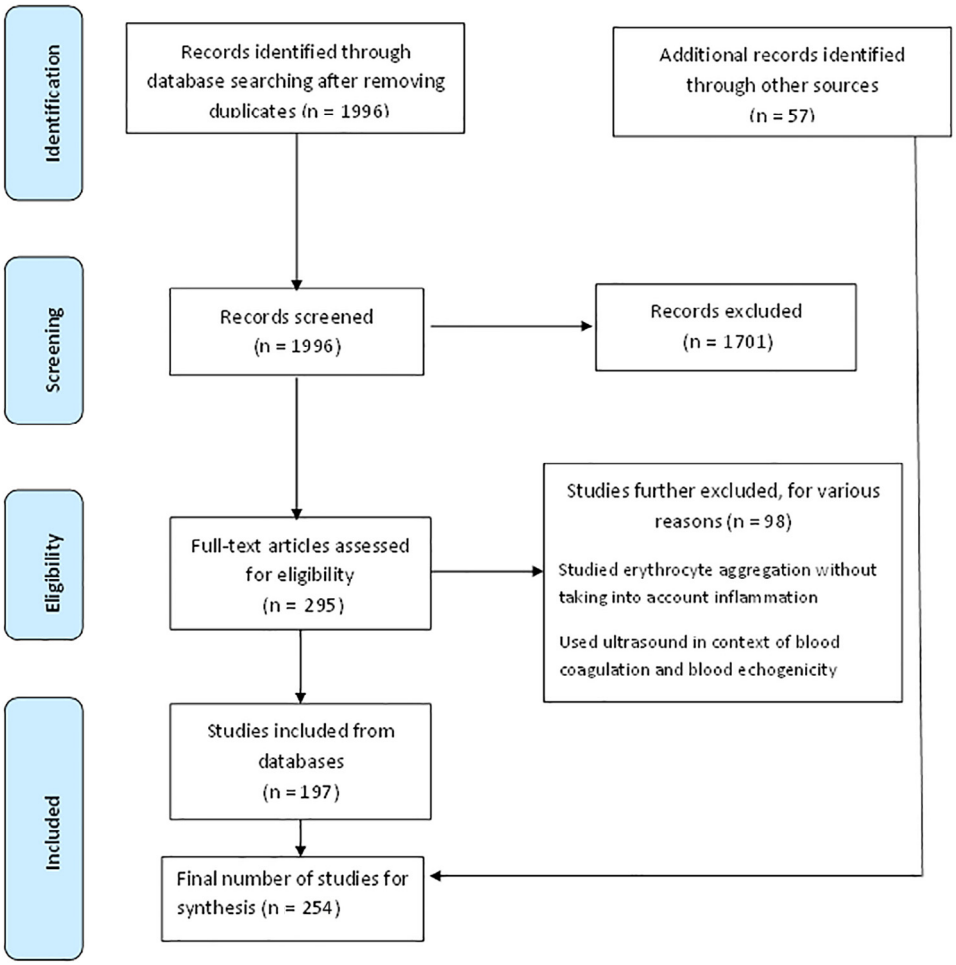

Fig. 1. Flow diagram indicating results of search and reasons for exclusion of studies.

effects of drugs on microcirculation and hemorheology by taking into account inflammation but not specifically erythrocyte aggregation. Thus, 197 articles were included for the final synthesis. Further, 57 articles were included from other sources mostly to set the study background (Fig. 1).

Description of studies

Erythrocyte aggregation and inflammation. The majority of retrieved studies correlated/associated inflammatory markers with elevated erythrocyte aggregation in several pathologic states (Almog et al. 2005; Ami et al. 2001; Baskurt et al. 1997; Berliner et al. 2000; Berliner et al. 2001; Brath et al. 2010; Czepiel et al. 2014; Duan et al. 2016; Elishkevitz et al. 2002; Fisher and Meiselman 1991; Gyawali and Richards 2014; Gyawali et al. 2014a; Justo et al. 2003; Kim et al. 2013; Krieger et al. 2004; Lakshmi et al. 2011; Lee et al. 2008; Maharshak et al. 2009; Novacek et al. 1996; Peled et al. 2008; Rogowski et al. 2000, 2005; Samocha-Bonet et al. 2003, 2004; Santos et al. 2011; Sargento et al. 2003; Schechner et al. 2003; Spengler et al. 2011; Toker et al. 2005; Vayá et al. 2011a, 2011b, 2013c; Wang et al. 2013; Woodward et al. 2003; Zeltser et al. 2004a, 2004b; Zilberman et al. 2005; Zimran et al. 2004). Although most studies measured acute-phase reactants as an inflammatory marker, few reports (n = 4) assayed pro-inflammatory cytokines as an inflammatory marker and correlated them with erythrocyte aggregation (Bornstein 2009; Hovhannisyan and Hovhanessyan 2009; Shenhar-Tsarfaty et al. 2008; Wang et al. 2013). In only 2 studies were histologic examinations of the tissue performed to find evidence of inflammation (Brath et al. 2010; Nemeth et al. 2014). The influence of acute-phase proteins on erythrocyte aggregation was also evaluated *in vitro* (Brust et al. 2014; Weng et al. 1996). Some studies noted significant reductions in erythrocyte aggregation and improved hemorheological profiles (Allegra et al. 1995; Nicolaides 2003) after lowering the inflammatory state through lifestyle modifications (Raz et al. 2007; Sandor

et al. 2014) or by the use of anti-inflammatory therapeutic agents (Ge et al. 2016; Jiang and Lian 2015; Kelly and Dominguez 2010; Li et al. 2015; Szentkereszty et al. 2014). Lastly, some studies (n = 15) underscored erythrocyte aggregation as a superior or independent marker of inflammation (Anuk et al. 2002; Assayag et al. 2005, 2008; Berliner et al. 2002, 2005; Gyawali et al. 2016; Levin et al. 2006; Maharshak et al. 2002; Rotstein et al. 2002a; Sharshun et al. 2003; Urbach et al. 2002, 2003, 2005; Vayá et al. 2013b) or sepsis (Yeom et al. 2017). Only 1 study reported increased erythrocyte aggregation with aging and associated it with increase in inflammation (Vayá et al. 2013a). Among all articles retrieved, 4 reported controversial findings (Adar et al. 2008; Nemeth et al. 2015; Sargento et al. 2005; Vayá et al. 2008). Table 1 summarizes publications that we considered related to erythrocyte aggregation and inflammation.

Erythrocyte aggregation and ultrasound imaging techniques. Several studies that have used ultrasound imaging techniques to characterize erythrocyte aggregation *in vitro* (Aggelopoulos et al. 1997; Alanen and Kormano 1985; Allard and Cloutier 1999; Allard et al. 1996; Boynard and Lelievre 1990; Cloutier and Qin 2000; Cloutier and Shung 1992; Cloutier et al. 1996, 2008; Franceschini et al. 2011; Garcia-Duitama et al. 2015; Haider et al. 2000, 2004; Huang 2009, 2010; Huang and Chang 2011; Huang et al. 2013, 2015; Kallio et al. 1989; Karabetsos et al. 1998; Khodabandehlou et al. 2002; Kim et al. 1989; Kitamura et al. 1995; Lupotti et al. 2004; Nam et al. 2008, 2009, 2012; Nguyen et al. 2008; Razavian et al. 1991, 1995; Rouffiac et al. 2002, 2003; Shehada et al. 1994; Shung and Paeng 2003; Sigel et al. 1982, 1983, 1984; Wang et al. 1992; Xu et al. 2010; Yu et al. 2009) and *in vivo* (Bok et al. 2015b; Cloutier et al. 1997; de Kroon et al. 1991; Fukushima et al. 2011; Kitamura and Kawasaki 1997; Li et al. 2011; Rouffiac et al. 2004; Sugata and Ito 2012; Wang and Shung 2001) were retrieved from the search. Erythrocyte aggregation determined by ultrasound means *in vitro* has been found to be the main cause of blood echogenicity seen in spontaneous echocardiographic contrast (Fatkin et al. 1997). It is also at the origin of flow phenomena known as the "black hole" and "collapsing ring" (Cao et al. 2001; Paeng et al. 2004; Qin et al. 1998; Shehada et al. 1994; Yuan and Shung 1989). To quantify the ultrasound measure and to provide interpretable physical indices of erythrocyte aggregation, Recchia and Wickline (1993) have used the integrated backscatter assessed with a clinical scanner; the measurement was expressed in decibels relative to a reference reflector. The absolute backscatter coefficient measured with an experimental ultrasound device was also used for this application (Yuan and Shung 1988a, 1988b). The modeling of received echoes from blood with descriptive statistical models has also been proposed (Cloutier et al. 2004; Destrempes et al. 2016; Huang 2011; Huang and Wang 2007). In the same way, the blood echogenicity, which was found to be higher among patients with claudication and venous thrombosis compared with normal controls, was reported to be decreased *in vitro* after the use of additive naftidrofuryl (a vasoactive substance with anti-aggregatory action) in blood (Alanen et al. 1990), validating the use of ultrasound signals to measure erythrocyte aggregation. All abovementioned approaches, although quantitative, do not describe erythrocyte aggregation in term of biophysical parameters and do not consider the spectral content of detected echoes for a better description of the phenomenon.

With the objective of providing such biophysical measures, Fontaine et al. (1999, 2002) considered constructive and destructive wave interference to describe the backscatter coefficient by modeling a structure factor $S(f)$, where f indicates frequency, and later, the same research group came up with an algorithm known as the structure factor size and attenuation estimator (SFSAE) (Franceschini et al. 2008, 2010; Yu and Cloutier 2007). Only 3 studies, so far, have used quantitative ultrasound to measure erythrocyte aggregation in relation to systemic inflammation, and all 3 came from the same laboratory (using SFSAE modeling) (Tripette et al. 2013, 2015; Yu et al. 2011). Studies using the SFSAE algorithm to measure erythrocyte aggregation in relation to inflammation are summarized in Table 2. Typical examples of SFSAE parametric images of erythrocyte aggregation with and without inflammation is presented in Figure 2.

Ultrasound imaging of systemic inflammation. We also selected studies that had used ultrasound techniques to study inflammation. Apart from references described above (Tripette et al. 2013; Yu et al. 2011), ultrasound methods to assess inflammation mostly used information from the structural damage or from the pathologic change of studied tissues and/or organs (Kristoffersen et al. 2006), which is not a direct measure of inflammation. Another ultrasound technique relies on injecting microbubbles with specific biological composition that bind with targeted receptors, cells or tissues, and then the signals generated from the complex are measured (Volz et al. 2016). However, the method requires venous access and does not seem to be suitable for serial assessment of inflammation because of the washout time for repetitive measures.

DISCUSSION

The present systematic review is the first of its kind that has endeavored to describe the importance of measuring erythrocyte aggregation as a marker of systemic inflammation. Precisely, the review emphasizes measurement of erythrocyte aggregation *in vivo* in real time continuously using quantitative imaging techniques. Among

Table 1. Studies associating inflammation with erythrocyte aggregation

	Study type	Comments
Correlations with inflammatory markers in various pathologies	*Cross-sectional studies* Almog et al. 2005 Ami et al. 2001 Assayag et al. 2005 Berliner et al. 2000 Czepiel et al. 2014 Elishkevitz et al. 2002 Gyawali and Richards 2014 Gyawali et al. 2014a, 2014b Justo et al. 2003 Krieger et al. 2004 Lakshmi et al. 2011 Maharshak et al. 2009 Novacek et al. 1996 Peled et al. 2008 Rogowski et al. 2000, 2005 Samocha-Bonet et al. 2003, 2004 Santos et al. 2011 Schechner et al. 2003 Shenhar-Tsarfaty et al. 2008 Spengler et al. 2011 Toker et al. 2005 Urbach et al. 2002 Vayá et al. 2011a, 2011b, 2013c Wang et al. 2013 Zeltser et al. 2004a, 2004b Zilberman et al. 2005 Zimran et al. 2004 *Prospective studies* Fisher and Meiselman 1991 Sargento et al. 2003 *Animal models* Baskurt et al. 1997 Brath et al. 2010 Duan et al. 2016	• Obesity (Samocha-Bonet et al. 2003, 2004; Vayá et al. 2011a) • Metabolic syndrome (Gyawali and Richards 2014; Gyawali et al. 2014b; Justo et al. 2003; Toker et al. 2005; Vayá et al. 2011b) • Inflammatory bowel disease (Maharshak et al. 2009; Novacek et al. 1996; Zilberman et al. 2005) • Atherothrombosis (Assayag et al. 2005; Berliner et al. 2001, 2005; Rogowski et al. 2005; Zeltser et al. 2004a) • Systemic lupus erythematosus (Santos et al. 2011; Spengler et al. 2011) • Myocardial infarction, ischemic stroke, coronary syndrome (Ami et al. 2001; Kim et al. 2013; Lakshmi et al. 2011; Lee et al. 2008; Sargento et al. 2003; Shenhar-Tsarfaty et al. 2008) • Obstructive sleep apnea (Peled et al. 2008) • Pelvic inflammatory disease (Almog et al. 2005) • Exposure to silica and benzopyrine (Duan et al. 2016) • Psoriasis (Vayá et al. 2013c) • Infection/sepsis (Ami et al. 2001; Baskurt et al. 1997; Berliner et al. 2000; Czepiel et al. 2014; Rogowski et al. 2000; Urbach et al. 2002) • Dyslipidemia (Schechner et al. 2003) • Gaucher's disease (Zimran et al. 2004) • Anemia with heart failure (Wang et al. 2013) • Aging (Vayá et al. 2013a) • Ischemia reperfusion (Brath et al. 2010) • Deep vein thrombosis (Krieger et al. 2004) • Population with and without cardiovascular diseases (Woodward et al. 2003) • Diabetes (Elishkevitz et al. 2002) • Cerebral ischemia (Fisher and Meiselman 1991)
Direct demonstration of the effect of acute-phase proteins on erythrocyte aggregation	*In vitro studies* Brust et al. 2014 Weng et al. 1996	• Effect of commercially available acute-phase proteins such as haptoglobin, C-reactive protein, ceruloplasmin, α_1-acid glycoprotein and α_1-antitrypsin were examined on aggregation kinetics determined by a laser aggregometer (Weng et al. 1996).
Decrease in erythrocyte aggregation due to associated decrease in inflammation through exercise and life style modifications	*Prospective studies* Raz et al. 2007 Sandor et al. 2014	• Weight loss after physical exercises/training and lifestyle modifications improved erythrocyte aggregation and decreased acute-phase proteins (Raz et al. 2007; Sandor et al. 2014).
Decrease in erythrocyte aggregation due to associated decrease in inflammation caused by use of therapeutic agents	*Case–control prospective studies* Jiang and Lian 2015 Li et al. 2015 *Animal models* Ge et al. 2016 Kelly and Dominguez 2010 Szentkereszty et al. 2014	• Danhong injection among patients with acute cerebral infarction improved hemorheological profile, which was attributed to the overall improvement in systemic inflammation (Jiang and Lian 2015). • Reducing inflammation with mycophenolate mofetil improved microvascular dysfunction and decreased erythrocyte aggregation (Kelly and Dominguez 2010).
Erythrocyte aggregation as an alternative or superior marker for assessing inflammation	*Cross-sectional studies* Assayag et al. 2005, 2008 Berliner et al. 2001, 2002 Gyawali et al. 2016 Maharshak et al. 2002 Rotstein et al. 2002a Urbach et al. 2003, 2005 *Prospective studies* Anuk et al. 2002 Levin et al. 2006 Sharshun et al. 2003 *Retrospective study* Vayá et al. 2013b *Animal model* Yeom et al. 2017	• Among acute-phase proteins compared, fibrinogen was shown to have the highest influence on erythrocyte aggregation, n = 234 (Assayag et al. 2005). • Among patients with ischemic stroke, n = 30 (Sharshun et al. 2003). • Among pediatric patients with infection and\or inflammation, n = 125 (Urbach et al. 2005). • Among patients with acute ischemic neurologic events, n = 60 (Anuk et al. 2002). • This study attempted to provide the cutoff for the erythrocyte aggregation value to discriminate low-grade inflammation in an apparently healthy population; however, the sample size was too low, n = 121 (Berliner et al. 2002). • Through use of the receiving operating characteristic curve, the study found that erythrocyte aggregation is a better marker than the inflammatory marker hsCRP (high-sensitivity C-reactive protein) and oxidative stress markers to classify patients with metabolic syndrome (Gyawali et al. 2016).
Erythrocyte aggregation not positively associated with inflammation	*Cross-sectional study* Vayá et al. 2008 *Prospective studies* Adar et al. 2008 Sargento et al. 2005 *Animal model* Nemeth et al. 2015	• Inflammatory markers were not correlated with erythrocyte aggregation. Authors argued that the low deformability of erythrocytes could be the probable reason as excessive rigidity would not allow the cells to aggregate properly (Vayá et al. 2008). • Hematocrit was not adjusted in this laboratory study, and this could be the reason for this unexpected finding (Sargento et al. 2005). • Inflammatory markers in Gaucher disease were not found to be correlated with erythrocyte aggregation indices, and the reason for increased erythrocyte aggregation was attributed to increased glucocerebroside levels in the red blood cells (Adar et al. 2008). • Erythrocyte aggregation was found to decrease unexpectedly in fulminant sepsis induced in an animal model (Nemeth et al. 2015) contrary to the established relationship between sepsis and erythrocyte aggregation. The use of *Escherichia coli* strain to induce sepsis (which can cause hemolysis) and/or methodological variations were put forward as possible reasons for these unexpected findings by the authors (Nemeth et al. 2015)

Table 2. Studies on quantitative ultrasound techniques that have used the SFSAE model for quantitative interpretation

Study	Study type	Comments
Tripette et al. (2015)	Case–control study, human participants (diabetic patients and healthy controls, n = 32)	Erythrocyte aggregation measured *in vivo* from the cephalic vein was found to be significantly higher in the diabetic population compared with normal controls. *Ex vivo* erythrocyte aggregation index measured with a laboratory erythroaggregometer was correlated with the reported SFSAE index.
Tripette et al. (2013)	Case–control interventional study; animal model (swine, n = 10)	Erythrocyte aggregation measured by ultrasound was found to be gradually increasing in the femoral vein during cardiopulmonary bypass surgery. Interleukin levels were found to be elevated only at the end of the procedure, whereas other markers of inflammation were less sensitive and did not exhibit any time evolution.
Yu et al. (2011)	Case–control interventional study; animal model (rabbit, n = 12)	Erythrocyte aggregation measured by ultrasound was found to be useful in evaluating deep-vein thrombosis risk profiling.

SFSAE = structure factor size and attenuation estimator.

the studies that have measured erythrocyte aggregation in the context of inflammation, the majority have correlated inflammatory markers with erythrocyte aggregation in several pathologies. Some studies have reported the decrease in erythrocyte aggregation after the use of anti-inflammatory therapeutic agents or lifestyle modifications. In addition, some studies have attempted to imply that erythrocyte aggregation is a superior marker of inflammation.

Abnormal erythrocyte aggregation is the tendency of an individual red blood cell to form a spherical clump, the size of which depends largely on the average size of normal rouleaux, erythrocyte surface free energy and bridging macromolecules in milieu (Bertoluzzo et al. 1999; Fabry 1987). Elevated erythrocyte aggregation has gained considerable attention over the last two decades because of its association with adverse cardiovascular outcomes and risk factors (Baskurt and Meiselman 2013; Fornal et al. 2008, 2009; Kesmarky et al. 2006; Urdulashvili et al. 2006). Inflammatory molecules have been reported to increase the rigidity of erythrocytes, change their physiologic shape and cause them to aggregate among themselves (Gyawali et al. 2012a, 2015). These molecules are believed to cause thrombi in the circulation at low flow shear stress through hyperviscous flow stagnation that may eventually lead to local ischemia (Baskurt and Meiselman 2003; Bishop et al. 2001; Ergun-Cagli et al. 2011; Gyawali et al. 2012b; Lacerda et al. 2017; Yedgar et al. 2002). Higher resi-

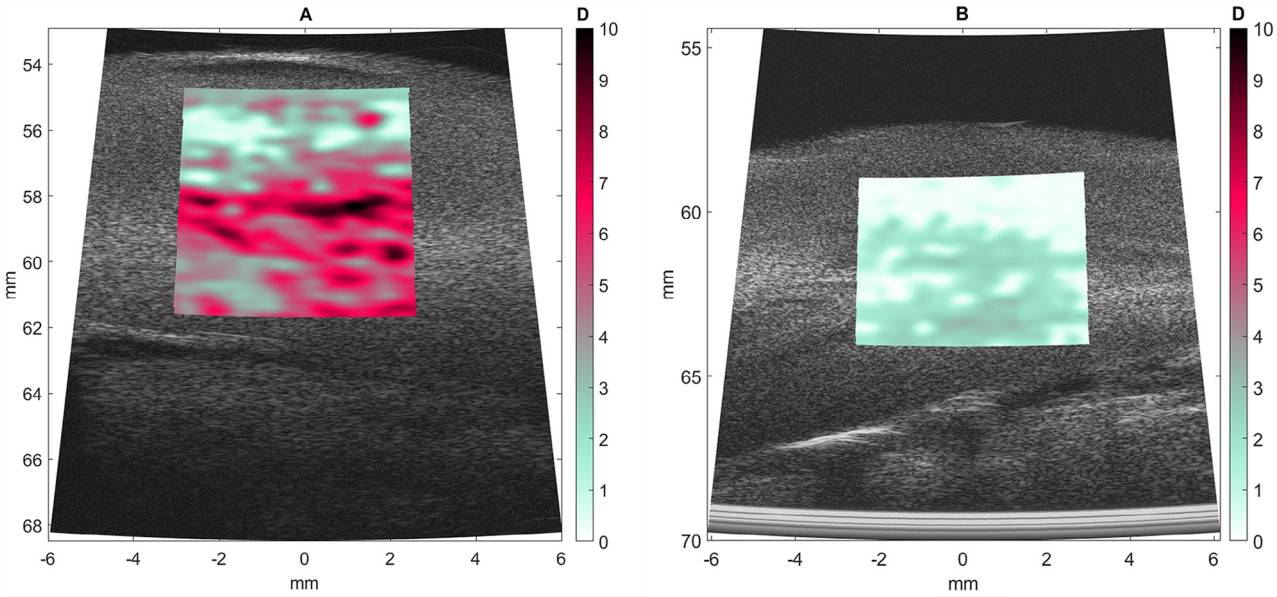

Fig. 2. Examples of structure factor size and attenuation estimator (SFSAE) parametric images of erythrocyte aggregation (*D*, longitudinal views) in cases of high (A) and low (B) inflammation. The ultrasound data were acquired from the femoral vein of a pig (experimental brain death model). The color maps vary from 0 (light cyan) to 10 (dark red). Parameter *D* has no unit (fractal dimension of spherical aggregates divided by the dimension of a single erythrocyte). The *y*-axis represents the depth of measurements; a surgical procedure was used to expose the vein, and degassed water was added in the cavity. The *x*-axis represents the lateral dimension of the image (unpublished data; the animal study received an institutional ethical committee approval from the University of Montreal Hospital Research Center).

dence time of erythrocytes in areas of viscous flow stagnation, promoted by low-shear aggregation, increases the interaction of other cellular and plasmatic elements with the endothelium (Watts et al. 2013). The presence of compact erythrocyte aggregates may further promote this process by increasing recruitment of platelets through erythrocyte–platelet interactions (Saniabadi et al. 1987; Valles et al. 2002). Activated platelets, through the release of prostaglandin E_2, further alter adjacent erythrocytes, reducing their filterability and mean cell volume (Li et al. 1996). Interaction of platelets with erythrocytes also increases the tendency of rouleaux formation (van Rooy and Pretorius 2016). Generation of endothelial dysfunction because of erythrocyte aggregates (Baskurt et al. 2004) may initiate cardiovascular events such as thrombosis, atherosclerosis and myocardial infarction. In fact, the odds of having myocardial infarction were reported to be 5.7 times higher among angina patients with elevated erythrocyte aggregation than in individuals with normal erythrocyte aggregation (Neumann et al. 1991).

Techniques for measuring erythrocyte aggregation

Several instruments have been developed to measure erythrocyte aggregation (Baskurt et al. 2009). These instruments rely mainly on the concept of laser light scattering and transmission (Baskurt et al. 2009; Pribush and Meyerstein 2007). Light transmission through a blood sample increases with increased erythrocyte aggregation; thus, changes in transmitted light are related to the size and conformation of aggregates (Gaspar-Rosas and Thurston 1988). Alterations in light reflectance or light transmittance versus time are plotted by laboratory-based aggregometers. Reflected or transmitted light is measured immediately after applying a high shear rate to disperse pre-existing aggregates; monitoring is then done after flow stoppage (Baskurt et al. 2009). The Myrenne aggregometer (Myrenne GmbH, Roetgen, Germany) (Baskurt et al. 2009), LORCA (Laser-Assisted Optical Rotational Cell Analyzer; RR Mechatronics, Hoorn, The Netherlands) (Hardeman et al. 2001) and RheoScan-A (Rheomeditech, Seoul, Korea) (Shin et al. 2009) are the three major instruments used for measuring erythrocyte aggregation for research purposes (Baskurt et al. 2009). Other laser-based Couette flow rheometers (Regulest formerly known as SEFAM, Nancy, France) (Stoltz et al. 1984), an He–Ne laser online erythrocyte aggregometer (Babu 2009; Babu and Singh 2004), digitized microscopic imaging (Chen et al. 1994; Foresto et al. 2000, 2002), computerized image analysis (Lacatusu et al. 2013), slide scanning of blood smear using an image analysis system (Assayag et al. 2005; Avitzour et al. 2003; Berliner et al. 2005; Rotstein et al. 2000, 2001b, 2002b; Samocha-Bonet et al. 2003) and a microfluidic approach (Yeom et al. 2017) have all been reported in the literature. An indirect measure of erythrocyte aggregation that is affected by the confounding effects of plasma viscosity and gravity is the erythrocyte sedimentation rate (ESR) (Potron et al. 1994; Rotstein et al. 2001a). Though the ESR has been widely associated with inflammatory diseases (Andresdottir et al. 2003), the information obtained from ESR assay is nonspecific and several other inflammatory markers have been found to be superior to this clinical approach for monitoring the course of infections and inflammatory diseases (Pecile et al. 2004).

Ultrasound imaging is the other promising approach for measuring erythrocyte aggregation. Though research on ultrasound for measuring erythrocyte aggregation has a long history (Boynard et al. 1987, 1988; Yuan and Shung 1988a, 1988b), these methods have not been widely accepted by hemorheologists and the clinical community in general, probably because of the complexity of acoustic modelling, quantitative approaches, data analyses, and the subjective nature of the image interpretation (*i.e.*, the lack of physical interpretability of reported data). Several attempts have been made to provide interpretable physical indices of erythrocyte aggregation to the scientific community and clinicians (Fontaine et al. 1999, 2002; Franceschini et al. 2008, 2010; Recchia and Wickline 1993; Sennaoui et al. 1997; Yu and Cloutier 2007). Mathematical models of ultrasound backscatter used to measure erythrocyte aggregation were developed for this purpose (Franceschini et al. 2013b; Mo and Cobbold 1986, 1992); a contemporary review of which has been done by Franceschini and Cloutier (2013a). Besides contributions from our team, a given number of research groups have published on the use of ultrasound methods to measure erythrocyte aggregation within the last 5 y (Bok et al. 2013, 2014, 2015b, 2016, 2017; Kong et al. 2013a, 2013b; Kurokawa et al. 2016; Ma et al. 2016; Nam et al. 2013; Sato and Watanabe 2013; Yeom and Lee 2015a, 2015b; Yeom et al. 2014, 2015), and some researchers have also provided a concept of photoacoustic imaging of erythrocyte aggregation (Bok et al. 2015a, 2017; Hysi et al. 2012a, 2012b; Saha and Kolios 2011). *In vivo* photoacoustic and photothermal cytometry have been reported to measure erythrocyte aggregation (Galanzha and Zharov 2011). These are promising alternatives to quantitative ultrasound that, however, have not been experimentally validated or applied in the context of inflammation monitoring.

Because quantitative ultrasound using the SFSAE model is the only method used *in vivo* to measure erythrocyte aggregation in the context of inflammation (Tripette et al. 2013, 2015; Yu et al. 2011), it seems a promising approach deserving additional validation. In the SFSAE model, backscattered echoes from erythrocytes are transformed to obtain a spectroscopic representation of the phenomenon from which biophysical parameters are extracted to determine properties of clustered erythrocytes.

This algorithm is based on the proven hypothesis that the spatial organization of erythrocytes is the main determinant of the ultrasound backscattered power when the backscatter cross section (*i.e.*, power backscattered by a single erythrocyte) and the hematocrit are known. Because erythrocyte aggregation modulates the spatial organization of individual red blood cells, $S(f)$ integrated into the SFSAE inverse problem algorithm can provide mean descriptors of the erythrocyte aggregation state by averaging, over time and space, echo properties of flowing blood (Franceschini et al. 2008, 2010; Yu and Cloutier 2007).

The general procedure of the SFSAE technique for measuring erythrocyte aggregation is briefly summarized here (Franceschini et al. 2008; Tripette et al. 2013, 2015; Yu et al. 2011). Ultrasound acquisitions are typically performed in frequent time intervals for continuous monitoring. The cephalic vein in the proximal portion of the forearm or the great saphenous vein in the distal portion of the leg is typically used. The ultrasound transducer is apposed on the skin to produce a longitudinal view of the vein. Venous monitoring is preferred because low shear rates offer favorable conditions for the formation and maintenance of aggregates. A hydraulic tourniquet can be placed downstream of the transducer to control the flow velocity (Garcia-Duitama et al. 2017). The targeted vein is typically scanned with a high-frequency ultrasound transducer (*e.g.,* with a 35-MHz central frequency probe) to acquire radiofrequency echoes. A spectral analysis of radiofrequency data is then used to compute the backscatter coefficient (BSC) of blood, and the SFSAE spectral model is fitted to the BSC. After minimization of the error between the experimental BSC and SFSAE spectral content, two descriptors of erythrocyte aggregation are obtained: W, known as the mean packing factor, is a dimensionless measure increasing proportionally with erythrocyte aggregation, and D is the mean aggregate diameter expressed in number of erythrocytes. It is the ratio of the diameter of a fractal isotropic aggregate to the diameter of one erythrocyte. D is typically smaller than 1 in the case of disaggregated erythrocytes. The SFSAE model compensates for skin and tissue ultrasound attenuations, allowing W and D to be independent of subject adiposity (as long as the radiofrequency signal-to-noise ratio is high enough to provide good estimates of the BSC).

Because the SFSAE providing a measure of the mean aggregate size is based on the modeling of the BSC, it comes with its own limitations. First, quantitative backscatter measurements require calibration on test phantoms (Oelze and Mamou 2016). However, a single measure may be necessary to calibrate the method for a given ultrasound instrumentation. Second, as the number of scatterers (*i.e.,* erythrocytes) increases, the backscattered echo magnitude is no longer linearly determined by their number because of constructive and destructive wave interference, as noticed for a long time from experiments on blood backscatter (Shung et al. 1976), and confirmed later with phantoms embedding different number densities of acoustic scatterers (Chen and Zagzebski 1996). Advantageously, the non-linear impact of the number density of scatterers on the backscatter coefficient is considered by the structure factor $S(f)$ of the SFSAE model (Yu and Cloutier 2007).

Importance of measuring erythrocyte aggregation in critical care

Although enhanced abnormal erythrocyte aggregation is a non-specific marker, it can be considered as a strong marker of inflammation generated by several clinical states and, thus, can potentially be used as a substitute for monitoring inflammation. An increased erythrocyte aggregation has been linked to the degree of organ failure in critically ill patients admitted to intensive care units (Reggiori et al. 2009). Likewise, hemorheological parameters including erythrocyte aggregation have been reported to be impaired among critically ill patients with or without infection (Kirschenbaum et al. 2000). More importantly, hemorheological parameters including erythrocyte aggregation have been found to predict mortality among critical care patients (Donadello et al. 2015; Totsimon et al. 2017) and erythrocyte aggregation has also been reported to predict unfavorable outcomes among patients undergoing percutaneous coronary interventions and among heart patients with diabetes (Jax et al. 2009; Steinvil et al. 2013).

The common technologies for measuring erythrocyte aggregation have mainly been developed in the context of laboratory instruments, and the basic physics behind them cannot support sizing of erythrocyte aggregates *in vivo* under flowing conditions within blood vessels. Also, these instruments need at least half a milliliter of an anticoagulated blood sample for measurement and, therefore, may not be suitable for continuous monitoring of inflammation in critical care units. However, the quantitative noninvasive spectroscopic ultrasound method described in the present review may offer the advantage of continuous monitoring of the inflammation state and, thus, may help in rapid decision making in a critical care setup, as suggested by Tripette et al. (2013). Therefore, the use of quantitative ultrasound erythrocyte aggregation imaging for prospective assessment of inflammatory states in clinical situations can be the one step forward in critical care medicine (Fernandes 2013).

Future perspectives

Unfavorable cardiovascular outcomes and cardiovascular risk factors are associated with enhanced erythrocyte aggregation (Gyawali et al. 2014b, 2016; Vayá et al. 1996; Wiewiora et al. 2013; Zannad et al. 1988), but it is still uncertain if the unfavorable outcomes are primarily due to the effect of erythrocyte hyperaggregation. In fact, find-

ings of several past and more contemporary studies suggest that the same underlying pathophysiological risk factors of cardiovascular diseases could be responsible for causing enhanced erythrocyte aggregation (Gyawali and Richards 2014; Gyawali et al. 2014a; Justo et al. 2003; Simmonds et al. 2016). Though it has not been proven that erythrocyte aggregation is a better indicator of systemic inflammation than other inflammatory mediator markers, the use of a non-invasive quantitative approach would definitely have the advantage of continuous measurement. The SFSAE approach to measuring erythrocyte aggregation seems promising, and proof-of-concept data are available (Cloutier et al. 2008; Tripette et al. 2013, 2015; Yu et al. 2011); nevertheless, extensive validation of the technique in the context of critical care patients remains to be performed. Larger clinical trials are required to establish the effect of measuring erythrocyte aggregation on clinical outcomes and choice of therapies.

CONCLUSIONS

Erythrocyte aggregation has been associated with systemic inflammation. Most of the techniques developed for measuring erythrocyte aggregation require at least half a milliliter of blood sample (invasive) and only work *in vitro*. However, recent improvements of the ultrasound SFSAE model may provide the capability of measuring the size, polydispersity and compactness of erythrocyte aggregates in real time, quantitatively and non-invasively (de Monchy et al. 2016). Thus, this technique favors repeated measurements of erythrocyte aggregation providing information on the aspect of systemic inflammation for appropriate patient management. Ultrasound assessment of erythrocyte aggregation can constitute a niche to predict uncontrolled inflammation that could lead to thrombotic complications, septic shock and multiple organ dysfunctions (Wan et al. 1997; Warltier et al. 2002). Therefore, the extent through which continuous monitoring of the inflammatory response in critical care may be useful through this approach to recognize early shocks and guide therapy requires a special attention.

Acknowledgments—Financial support was provided by the Canadian Institutes of Health Research (MOP-84358, CPG-151959) and the Natural Sciences and Engineering Research Council of Canada (CHRP-508337-17).

REFERENCES

Adar T, Ami RB, Elstein D, Zimran P, Berliner S, Yedgar S, Barshtein G. Increased red blood cell aggregation in patients with Gaucher disease is non-inflammatory. Clin Hemorheol Microcirc 2008;40:113–118.

Aggelopoulos EG, Karabetsos E, Koutsouris D. In vitro estimation of red blood cells' aggregation using ultrasound Doppler techniques. Clin Hemorheol Microcirc 1997;17:107–115.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.

Alanen A, Kormano M. Ultrasonic echoes registered from erythrocytes. Invest Radiol 1985;20:521–524.

Alanen A, Kallio T, Lehto I, Wiernsperger N. The effect of naftidrofuryl on red blood cell aggregation detected in vitro with ultrasound. J Cardiovasc Pharmacol 1990;16:S33–S35.

Allard L, Cloutier G. Power Doppler ultrasound scan imaging of the level of red blood cell aggregation: An in vitro study. J Vasc Surg 1999;30:157–168.

Allard L, Cloutier G, Durand LG. Effect of the insonification angle on the Doppler backscattered power under red blood cell aggregation conditions. IEEE Trans Ultrason Ferroelectr Freq Control 1996;43:211–219.

Allegra C, Bartolo M, Jr., Carioti B, Cassiani D. An original microhaemorheological approach to the pharmacological effects of Daflon 500 mg in severe chronic venous insufficiency. Int J Microcirc Clin Exp 1995;15(1 Suppl):50–54.

Almog B, Gamzu R, Almog R, Lessing JB, Shapira I, Berliner S, Pauzner D, Maslovitz S, Levin I. Enhanced erythrocyte aggregation in clinically diagnosed pelvic inflammatory disease. Sex Transm Dis 2005;32:484–486.

Ami RB, Barshtein G, Zeltser D, Goldberg Y, Shapira I, Roth A, Keren G, Miller H, Prochorov V, Eldor A, Berliner S, Yedgar S. Parameters of red blood cell aggregation as correlates of the inflammatory state. Am J Physiol Heart Circ Physiol 2001;280:H1982–H1988.

Andresdottir MB, Sigfusson N, Sigvaldason H, Gudnason V. Erythrocyte sedimentation rate, an independent predictor of coronary heart disease in men and women: The Reykjavik Study. Am J Epidemiol 2003;158:844–851.

Anuk T, Assayag EB, Rotstein R, Fusman R, Zeltser D, Berliner S, Avitzour D, Shapira I, Arber N, Bornstein NM. Prognostic implications of admission inflammatory profile in acute ischemic neurological events. Acta Neurol Scand 2002;106:196–199.

Asimakopoulos G. Systemic inflammation and cardiac surgery: An update. Perfusion 2001;16:353–360.

Assayag EB, Bornstein N, Shapira I, Mardi T, Goldin Y, Tolshinski T, Vered Y, Zakuth V, Burke M, Berliner S, Bonet DS. Inflammation-sensitive proteins and erythrocyte aggregation in atherothrombosis. Int J Cardiol 2005;98:271–276.

Assayag EB, Bova I, Kesler A, Berliner S, Shapira I, Bornstein NM. Erythrocyte aggregation as an early biomarker in patients with asymptomatic carotid stenosis. Dis Markers 2008;24:33–39.

Avitzour D, Shapira I, Rotstein R, Zeltser D, Mardi T, Justo D, Rozenblat M, Berliner S. Your lab focus: Science. Image analysis of erythrocyte adhesiveness/aggregation. Lab Med 2003;34:213–216.

Babu N. Alterations in aggregation parameters of erythrocytes due to hyper cholesterol in type-2 diabetes mellitus. Open Circ Vasc J 2009;2:10–14.

Babu N, Singh M. Influence of hyperglycemia on aggregation, deformability and shape parameters of erythrocytes. Clin Hemorheol Microcirc 2004;31:27–80.

Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost 2003;29:435–450.

Baskurt OK, Meiselman HJ. Erythrocyte aggregation: Basic aspects and clinical importance. Clin Hemorheol Microcirc 2013;53:23–37.

Baskurt OK, Temiz A, Meiselman HJ. Red blood cell aggregation in experimental sepsis. J Lab Clin Med 1997;130:183–190.

Baskurt OK, Yalcin O, Ozdem S, Armstrong JK, Meiselman HJ. Modulation of endothelial nitric oxide synthase expression by red blood cell aggregation. Am J Physiol Heart Circ Physiol 2004;286:H222–H229.

Baskurt OK, Uyuklu M, Ulker P, Cengiz M, Nemeth N, Alexy T, Shin S, Hardeman MR, Meiselman HJ. Comparison of three instruments for measuring red blood cell aggregation. Clin Hemorheol Microcirc 2009;43:283–298.

Berliner AS, Shapira I, Rogowski O, Sadees N, Rotstein R, Fusman R, Avitzour D, Cohen S, Arber N, Zeltser D. Combined leukocyte and erythrocyte aggregation in the peripheral venous blood during sepsis:

An indication of commonly shared adhesive protein(s). Int J Clin Lab Res 2000;30:27–31.
Berliner S, Zeltser D, Rotstein R, Fusman R, Shapira I. A leukocyte and erythrocyte adhesiveness/aggregation test to reveal the presence of smoldering inflammation and risk factors for atherosclerosis. Med Hypotheses 2001;57:207–209.
Berliner S, Zeltser D, Shapira I, Assayag EB, Mardi T, Serov J, Aharonov S, Arber N, Rotstein R. A simple biomarker to exclude the presence of low grade inflammation in apparently healthy individuals. J Cardiovasc Risk 2002;9:281–286.
Berliner S, Rogowski O, Aharonov S, Mardi T, Tolshinsky T, Rozenblat M, Justo D, Deutsch V, Serov J, Shapira I, Zeltzer D. Erythrocyte adhesiveness/aggregation: A novel biomarker for the detection of low-grade internal inflammation in individuals with atherothrombotic risk factors and proven vascular disease. Am Heart J 2005;149:26–267.
Bertoluzzo SM, Bollini A, Rasia M, Raynal A. Kinetic model for erythrocyte aggregation. Blood Cells Mol Dis 1999;25:339–349.
Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. Effect of erythrocyte aggregation on velocity profiles in venules. Am J Physiol Heart Circ Physiol 2001;280:H222–H236.
Bok TH, Nam KH, Paeng DG, Kim J. Probability distribution variation in high-frequency ultrasound blood echogenicity under in-vitro and in-vivo blood flow. Proc Mtgs Acoust 2013;19:075084.
Bok TH, Hysi E, Kolios MC. Simultaneous measurement of erythrocyte aggregarion and oxygen saturation under in vitro pulsatile blood flow by high-frequency photoacoustics. Proc IEEE Int Ultrason Symp 2014;1292–1295.
Bok TH, Hysi E, Kolios MC. High-frequency photoacoustic imaging of erythrocyte aggregation and oxygen saturation: Probing hemodynamic relations under pulsatile blood flow. Proc SPIE 2015a; 9323(93231Q):7.
Bok TH, Li Y, Nam KH, Choi JC, Paeng DG. Feasibility study of high-frequency ultrasonic blood imaging in human radial artery. J Med Biol Eng 2015b;35:21–27.
Bok TH, Hysi E, Kolios MC. Simultaneous assessment of red blood cell aggregation and oxygen saturation under pulsatile flow using high-frequency photoacoustics. Biomed Opt Express 2016;7:2769–2780.
Bok TH, Hysi E, Kolios MC. Quantitative photoacoustic assessment of red blood cell aggregation under pulsatile blood flow: Experimental and theoretical approaches. Proc SPIE 2017;10064:100645F.
Bornstein NM. Erythrocyte aggregation as an early predictor for 1 year survival following acute ischemic stroke. Cerebrovasc Dis 2009; 27(5 Suppl):181–209.
Boynard M, Lelievre JC. Size determination of red blood cell aggregates induced by dextran using ultrasound backscattering phenomenon. Biorheology 1990;27:39–46.
Boynard M, Lelievre JC, Guillet R. Aggregation of red blood cells studied by ultrasound backscattering. Biorheology 1987;24:451–461.
Boynard M, Razavian M, Beuzard Y. Evaluation of sickle cell aggregation by ultrasound backscattering. Clin Hemorheol 1988;8:687–694.
Brath E, Nemeth N, Kiss F, Sajtos E, Hever T, Matyas L, Toth L, Miko I, Furka I. Changes of local and systemic hemorheological properties in intestinal ischemia-reperfusion injury in the rat model. Microsurgery 2010;30:321–326.
Brenner DR, Scherer D, Muir K, Schildkraut J, Boffetta P, Spitz MR, Le Marchand L, Chan AT, Goode EL, Ulrich CM, Hung RJ. A review of the application of inflammatory biomarkers in epidemiologic cancer research. Cancer Epidemiol Biomarkers Prev 2014;23:1729.
Brust M, Aouane O, Thiebaud M, Flormann D, Verdier C, Kaestner L, Laschke MW, Selmi H, Benyoussef A, Podgorski T, Coupier G, Misbah C, Wagner C. The plasma protein fibrinogen stabilizes clusters of red blood cells in microcapillary flows. Sci Rep 2014;4: 4348.
Cao PJ, Paeng DG, Shung KK. The "black hole" phenomenon in ultrasonic backscattering measurement under pulsatile flow with porcine whole blood in a rigid tube. Biorheology 2001;38:15–26.
Chen JF, Zagzebski JA. Frequency dependence of backscatter coefficient versus scatterer volume fraction. IEEE Trans Ultrason Ferroelectr Freq Control 1996;43:345–353.
Chen S, Barshtein G, Gavish B, Mahler Y, Yedgar S. Monitoring of red blood cell aggregability in a flow-chamber by computerized image analysis. Clin Hemorheol Microcirc 1994;14:497–508.
Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907–916.
Cloutier G, Qin Z. Shear rate dependence of ultrasound backscattering from blood samples characterized by different levels of erythrocyte aggregation. Ann Biomed Eng 2000;28:399–407.
Cloutier G, Shung K. Study of red cell aggregation in pulsatile flow from ultrasonic Doppler power measurements. Biorheology 1992;30:443–461.
Cloutier G, Qin Z, Durand LG, Teh BG. Power Doppler ultrasound evaluation of the shear rate and shear stress dependences of red blood cell aggregation. IEEE Trans Biomed Eng 1996;43:441–450.
Cloutier G, Weng X, Roederer GO, Allard L, Tardif F, Beaulieu R. Differences in the erythrocyte aggregation level between veins and arteries of normolipidemic and hyperlipidemic individuals. Ultrasound Med Biol 1997;23:1383–1393.
Cloutier G, Daronat M, Savéry D, Garcia D, Durand LG, Foster FS. Non-Gaussian statistics and temporal variations of the ultrasound signal backscattered by blood at frequencies between 10 and 58 MHz. J Acoust Soc Am 2004;116:566–577.
Cloutier G, Zimmer A, Yu FT, Chiasson JL. Increased shear rate resistance and fastest kinetics of erythrocyte aggregation in diabetes measured with ultrasound. Diabetes Care 2008;31:1400–1402.
Czepiel J, Jurczyszyn A, Biesiada G, Sobczyk-Krupiarz I, Jałowiecka I, Świstek M, Perucki W, Teległów A, Marchewka J Dąbrowski Z. Rheological properties of erythrocytes in patients infected with *Clostridium difficile*. Postepy Hig Med Dosw (Online) 2014;68:1397–1405.
de Kroon MG, Slager CJ, Gussenhoven WJ, Serruys PW, Roelandt JR, Bom N. Cyclic changes of blood echogenicity in high-frequency ultrasound. Ultrasound Med Biol 1991;17:723–728.
de Monchy R, Chayer B, Cloutier G, Franceschini E. Effective medium theory combined with a polydisperse structure factor model for characterizing red blood cell aggregation. Proc IEEE Int Ultrason Symp 2016;doi:10.1109/ULTSYM.2016.7728606.
Destrempes F, Franceschini E, Yu FT, Cloutier G. Unifying concepts of statistical and spectral quantitative ultrasound techniques. IEEE Trans Med Imaging 2016;35:488–500.
Donadello K, Piagnerelli M, Reggiori G, Gottin L, Scolletta S, Occhipinti G, Zouaoui Boudjeltia K, Vincent JL. Reduced red blood cell deformability over time is associated with a poor outcome in septic patients. Microvasc Res 2015;101:8–14.
Duan J, Yu Y, Li Y, Wang Y, Sun Z. Inflammatory response and blood hypercoagulable state induced by low level co-exposure with silica nanoparticles and benzo[a]pyrene in zebrafish (*Danio rerio*) embryos. Chemosphere 2016;151:152–162.
Elishkevitz K, Fusman R, Koffler M, Shapira I, Zeltser D, Avitzour D, Arber N, Berliner S, Rotstein R. Rheological determinants of red blood cell aggregation in diabetic patients in relation to their metabolic control. Diabet Med 2002;19:152–156.
Ergun-Cagli K, Ileri-Gurel E, Ozeke O, Seringec N, Yalcinkaya A, Kocabeyoglu S, Basar FN, Sen N, Cagli K Dikmenoglu N. Blood viscosity changes in slow coronary flow patients. Clin Hemorheol Microcirc 2011;47:27–35.
Fabry T. Mechanism of erythrocyte aggregation and sedimentation. Blood 1987;70:1572–1576.
Fatkin D, Loupas T, Low J, Feneley M. Inhibition of red cell aggregation prevents spontaneous echocardiographic contrast formation in human blood. Circulation 1997;96:889–896.
Fernandes CJ, Jr. The future has arrived. Crit Care Med 2013;41:2062–2063.
Fisher M, Meiselman HJ. Hemorheological factors in cerebral ischemia. Stroke 1991;22:1164–1169.
Fontaine I, Bertrand M, Cloutier G. A system-based approach to modeling the ultrasound signal backscattered by red blood cells. Biophys J 1999;77:2387–2399.
Fontaine I, Savery D, Cloutier G. Simulation of ultrasound backscattering by red cell aggregates: Effect of shear rate and anisotropy. Biophys J 2002;82:1696–1710.

Foresto P, D'Arrigo M, Carreras L, Cuezzo RE, Valverde J Rasia R. Evaluation of red blood cell aggregation in diabetes by computarized image analysis. Medicina 2000;60:570–572.
Foresto P, D'Arrigo M, Racca L, Filippini F, Gallo R, Valverde J Rasia RJ. Comparative analysis of aggregate shapes by digitized microscopic images. Application to hypertension. Clin Hemorheol Microcirc 2002;26:137.
Fornal M, Korbut RA, Lekka M, Pyka-Fosciak G, Wizner B, Styczen J, Grodzicki T. Rheological properties of erythrocytes in patients with high risk of cardiovascular disease. Clin Hemorheol Microcirc 2008; 39:213–219.
Fornal M, Korbut RA, Krolczyk J, Grodzicki T. Left ventricular geometry and rheological properties of erythrocytes in patients at cardiovascular disease risk. Clin Hemorheol Microcirc 2009;43: 203–208.
Franceschini E, Cloutier G. Modeling of ultrasound backscattering by aggregating red blood cells. In: Mamou J, Oelze M, (eds). Quantitative ultrasound in soft tissues. Dordrecht: Springer; 2013a. p. 117–145.
Franceschini E, Yu FT, Cloutier G. Simultaneous estimation of attenuation and structure parameters of aggregated red blood cells from backscatter measurements. J Acoust Soc Am 2008;123:EL85–EL91.
Franceschini E, Yu FT, Destrempes F, Cloutier G. Ultrasound characterization of red blood cell aggregation with intervening attenuating tissue-mimicking phantoms. J Acoust Soc Am 2010;127:1104–1115.
Franceschini E, Metzger B, Cloutier G. Forward problem study of an effective medium model for ultrasound blood characterization. IEEE Trans Ultrason Ferroelectr Freq Control 2011;58:2668–2679.
Franceschini E, Saha RK, Cloutier G. Comparison of three scattering models for ultrasound blood characterization. IEEE Trans Ultrason Ferroelectr Freq Control 2013b;60:2321–2334.
Freer G, Rindi L. Intracellular cytokine detection by fluorescence-activated flow cytometry: Basic principles and recent advances. Methods 2013;61:30–38.
Fukushima T, Hasegawa H, Kanai H. Estimation of scatterer diameter by normalized power spectrum of high-frequency ultrasonic RF echo for assessment of red blood cell aggregation. Jpn J Appl Phys 2011; 50:07HF02.
Galanzha EI, Zharov VP. In vivo photoacoustic and photothermal cytometry for monitoring multiple blood rheology parameters. Cytometry A 2011;79:746–757.
Garcia-Duitama J, Chayer B, Han A, Garcia D, Oelze ML, Cloutier G. Experimental application of ultrafast imaging to spectral tissue characterization. Ultrasound Med Biol 2015;41:2506–2519.
Garcia-Duitama J, Chayer B, Garcia D, Goussard Y, Cloutier G. Protocol for robust *in vivo* measurements of erythrocyte aggregation using ultrasound spectroscopy. Ultrasound Med Biol 2017;43:2871–2881.
Gaspar-Rosas A, Thurston G. Erythrocyte aggregate rheology by transmitted and reflected light. Biorheology 1988;25:471.
Ge YB, Wang ZG, Xiong Y, Huang XJ, Mei ZN, Hong ZG. Anti-inflammatory and blood stasis activities of essential oil extracted from *Artemisia argyi* leaf in animals. J Nat Med 2016;70:531–538.
Goris RJA, te Boekhorst TP, Nuytinck JK, Gimbrère JS. Multiple-organ failure: Generalized autodestructive inflammation? Arch Surg 1985;120:1109–1115.
Gustot T. Multiple organ failure in sepsis: Prognosis and role of systemic inflammatory response. Curr Opin Crit Care 2011;17:153–159.
Gyawali P, Richards RS. Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome. Redox Rep 2014;20:139–144.
Gyawali P, Richards RS, Nwose EU. Erythrocyte morphology in metabolic syndrome. Expert Rev Hematol 2012a;5:523–531.
Gyawali P, Richards RS, Nwose EU Bwititi PT. Whole-blood viscosity and metabolic syndrome. Clin Lipidol 2012b;7:709–719.
Gyawali P, Richards RS, Hughes DL, Tinley P. Erythrocyte aggregation and metabolic syndrome. Clin Hemorheol Microcirc 2014a;57: 73–83.
Gyawali P, Richards RS, Tinley P, Nwose EU. Hemorheology, Ankle Brachial Pressure Index (ABPI) and Toe Brachial Pressure Index (TBPI) in metabolic syndrome. Microvasc Res 2014b;95:31–36.
Gyawali P, Richards RS, Bwititi PT, Nwose EU. Association of abnormal erythrocyte morphology with oxidative stress and inflammation in metabolic syndrome. Blood Cells Mol Dis 2015;54:360–363.
Gyawali P, Richards RS, Tinley P, Nwose EU, Bwititi PT. Hemorheological parameters better classify metabolic syndrome than novel cardiovascular risk factors and peripheral vascular disease marker. Clin Hemorheol Microcirc 2016;64:1–5.
Haider L, Snabre P, Boynard M. Rheo-acoustical study of the shear disruption of reversible aggregates: Ultrasound scattering from concentrated suspensions of red cell aggregates. J Acoust Soc Am 2000;107:1715–1726.
Haider L, Snabre P Boynard M. Rheology and ultrasound scattering from aggregated red cell suspensions in shear flow. Biophys J 2004;87: 2322–2334.
Hardeman M, Dobbe J, Ince C. The laser-assisted optical rotational cell analyzer (LORCA) as red blood cell aggregometer. Clin Hemorheol Microcirc 2001;25:1–12.
Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444:860–867.
Hovhannisyan IG, Hovhanessyan RA. The correlation between interleukin IL-1 beta and the blood aggregative state of acute ischemic stroke. Eur J Neurol 2009;16(3 Suppl):395, P2172.
Huang CC. Cyclic variations of high-frequency ultrasonic backscattering from blood under pulsatile flow. IEEE Trans Ultrason Ferroelectr Freq Control 2009;56:1677–1688.
Huang CC. High-frequency attenuation and backscatter measurements of rat blood between 30 and 60 MHz. Phys Med Biol 2010;55:5801–5815.
Huang CC. Detecting spatial variations of erythrocytes by ultrasound backscattering statistical parameters under pulsatile flow. IEEE Trans Biomed Eng 2011;58:1163–1171.
Huang CC, Chang YC. Ultrasonic attenuation and backscatter from flowing whole blood are dependent on shear rate and hematocrit between 10 and 50 MHz. IEEE Trans Ultrason Ferroelectr Freq Control 2011;58:357–368.
Huang CC, Wang SH. Statistical variations of ultrasound signals backscattered from flowing blood. Ultrasound Med Biol 2007;33: 1943–1954.
Huang CC, Liao CC, Lee PY, Shih CC. The effect of flow acceleration on the cyclic variation of blood echogenicity under pulsatile flow. Ultrasound Med Biol 2013;39:670–680.
Huang CC, Chou HL, Chen PY. Measurement of the Doppler power of flowing blood using ultrasound Doppler devices. Ultrasound Med Biol 2015;41:565–573.
Hysi E, Saha RK, Kolios MC. On the use of photoacoustics to detect red blood cell aggregation. Biomed Opt Express 2012a;3:2326–2338.
Hysi E, Saha RK, Kolios MC. Photoacoustic ultrasound spectroscopy for assessing red blood cell aggregation and oxygenation. J Biomed Opt 2012b;17:125006, 1-06-10.
Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, Kremastinos DT. Inflammatory and non-invasive vascular markers: The multimarker approach for risk stratification in coronary artery disease. Atherosclerosis 2008;199:3–11.
Jax TW, Peters AJ, Plehn G, Schoebel FC. Hemostatic risk factors in patients with coronary artery disease and type 2 diabetes: A two year follow-up of 243 patients. Cardiovasc Diabetol 2009;8:48.
Jiang Y, Lian Y. Effects of Danhong injection on hemodynamics and the inflammation-related NF-κB signaling pathway in patients with acute cerebral infarction. Genet Mol Res 2015;14:16929–16937.
Justo D, Marilus R, Mardi T, Tolchinsky T, Goldin Y, Rozenblat M, Rogowski O, Yerushalmi Y, Stern N, Shenkerman G. The appearance of aggregated erythrocytes in the peripheral blood of individuals with insulin resistance. Diabetes Metab Res Rev 2003;19:386–391.
Kallio T, Alanen A, Kormano M. The in vitro echogenicity of flowing blood in patients with vascular disease and the effect of naftidrofuryl. Ultrasound Med Biol 1989;15:555–559.

Karabetsos E, Papaodysseus C, Koutsouris D. Design and development of a new ultrasonic doppler technique for estimation of the aggregation of red blood cells. Measurement 1998;24:207–215.
Kelly K, Dominguez JH. Treatment of the post-ischaemic inflammatory syndrome of diabetic nephropathy. Nephrol Dial Transplant 2010; 25:3204–3212.
Kesmarky G, Feher G, Koltai K, Horvath B, Toth K. Viscosity, hemostasis and inflammation in atherosclerotic heart diseases. Clin Hemorheol Microcirc 2006;35:67–73.
Khodabandehlou T, Boynard M, Guillet R, Devehat CL. Sensitivity of the ultrasonic interferometry method (Echo-Cell) to changes of red cell aggregation: Application to diabetes. Clin Hemorheol Microcirc 2002;27:219–232.
Kim J, Chung H, Cho M, Lee BK, Karimi A, Shin S. The role of critical shear stress on acute coronary syndrome. Clin Hemorheol Microcirc 2013;55:101–109.
Kim SY, Miller IF, Sigel B, Consigny PM, Justin J. Ultrasonic evaluation of erythrocyte aggregation dynamics. Biorheology 1989;26: 723–736.
Kirschenbaum LA, Aziz M, Astiz ME, Saha DC, Rackow EC. Influence of rheologic changes and platelet-neutrophil interactions on cell filtration in sepsis. Am J Respir Crit Care Med 2000;161:1602–1607.
Kitamura H, Kawasaki S. Detection and clinical significance of red cell aggregation in the human subcutaneous vein using a high-frequency transducer (10 MHz): A preliminary report. Ultrasound Med Biol 1997; 23:933–938.
Kitamura H, Sigel B, Machi J, Feleppa EJ, Sokil-Melgar J, Kalisz A, Justin J. Roles of hematocrit and fibrinogen in red cell aggregation determined by ultrasonic scattering properties. Ultrasound Med Biol 1995;21:827–832.
Kong Q, Nam KH, Paeng DG. A simulation model of cyclic variation of red blood cell aggregation under Couette and Poiseuille flows. J Acoust Soc Am 2013a;134:4122.
Kong Q, Nam KH, Paeng DG, Li Y. 2013 The computer simulation of microscopic interactions of RBC aggregation based on the depletion model under pulsatile flow. Proc IEEE Int Ultrason Symp 2013b; 1749–1752.
Krieger E, van Der Loo B, Amann-Vesti BR, Rousson V, Koppensteiner R. C-Reactive protein and red cell aggregation correlate with late venous function after acute deep venous thrombosis. J Vasc Surg 2004; 40:644–649.
Kristoffersen H, Torp-Pedersen S, Terslev L, Qvistgaard E, Cato Holm C, Ellegaard K, Bliddal H. Indications of inflammation visualized by ultrasound in osteoarthritis of the knee. Acta Radiol 2006;47: 281–286.
Kurokawa Y, Taki H, Yashiro S, Nagasawa K, Ishigaki Y, Kanai H. Estimation of size of red blood cell aggregates using backscattering property of high-frequency ultrasound: In vivo evaluation. Jpn J Appl Phys 2016;55:07 KF12.
Lacatusu D, Caruntu ID, Rusu V. Study on erythrocyte aggregation using computerized image analysis methods. Rev Med Chir Soc Med Nat Iasi 2013;117:801–805.
Lacerda FH, de Mattos Mendes de Almeida VB, Nunes JT, de Carvalho Melo Junior JA, Park M. Routine ultrasound-guided central venous access catheterization: A window to new findings! J Crit Care 2017;37:262–263.
Lakshmi AB, Uma P, Venkatachalam C, Rao GSN. A simple slide test to assess erythrocyte aggregation in acute ST-elevated myocardial infarction and acute ischemic stroke: Its prognostic significance. Indian J Pathol Microbiol 2011;54:63–69.
Lee BK, Durairaj A, Mehra A, Wenby RB, Meiselman HJ, Alexy T. Hemorheological abnormalities in stable angina and acute coronary syndromes. Clin Hemorheol Microcirc 2008;39:43–51.
Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. J Gerontol A Biol Sci Med Sci 2008; 63:879–884.
Levin I, Helpman L, Maslovitz S, Pauzner D, Shapira I, Gamzu R, Almog B. Erythrocyte aggregation is increased in preterm premature rupture of the membranes. Eur J Obstet Gynecol Reprod Biol 2006;125: 199–201.
Li LJ, Li YM, Qiao BY, Jiang S, Li X, Du HM, Han PC, Shi J. The value of Safflower Yellow Injection for the treatment of acute cerebral infarction: A randomized controlled trial. Evid Based Complement Alternat Med 2015;2015:478793.
Li Q, Jungmann V, Kiyatkin A, Low PS. Prostaglandin E2 stimulates a Ca^{2+}-dependent K^+ channel in human erythrocytes and alters cell volume and filterability. J Biol Chem 1996;271:18651–18656.
Li Y, Bok TH, Yang JH, Choi MJ, Paeng DG. The acute effects of smoking on the cyclic variations in blood echogenicity of carotid artery. Ultrasound Med Biol 2011;37:513–521.
Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–1143.
Lupotti FA, Zimmer A, Daronat M, Foster FS, van Der Steen AF, Cloutier G. Effects of aggregation of red cells and linear velocity gradients on the correlation-based method for quantitative IVUS blood flow at 20 MHz. Ultrasound Med Biol 2004;30:205–214.
Ma X, Huang B, Wang G, Fu X, Qiu S. Numerical simulation of the red blood cell aggregation and deformation behaviors in ultrasonic field. Ultrason Sonochem 2016;38:604–613.
Maharaj C, Laffey JG. New strategies to control the inflammatory response in cardiac surgery. Curr Opin Anesthesiol 2004;17:35–48.
Maharshak N, Shapira I, Rotstein R, Serov J, Aharonov S, Mardi T, Twig A, Rubinstein A, Kofler M, Berliner S. The erythrocyte adhesiveness/aggregation test for the detection of an acute phase response and for the assessment of its intensity. Clin Lab Haematol 2002;24:205–210.
Maharshak N, Arbel Y, Shapira I, Berliner S, Ami RB, Yedgar S, Barshtein G, Dotan I. Increased strength of erythrocyte aggregates in blood of patients with inflammatory bowel disease. Inflamm Bowel Dis 2009;15:707–713.
Mantovani A. Cancer: Inflammation by remote control. Nature 2005; 435:752–753.
McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord 2004;10:S3–S7.
Mo LY, Cobbold RS. A stochastic model of the backscattered Doppler ultrasound from blood. IEEE Trans Biomed Eng 1986;33:20–27.
Mo LYL, Cobbold RSC. A unified approach to modeling the backscattered Doppler ultrasound from blood. IEEE Trans Biomed Eng 1992;39: 450–461.
Muckart DJ, Bhagwanjee S. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of the systemic inflammatory response syndrome and allied disorders in relation to critically injured patients. Crit Care Med 1997; 25:1789–1795.
Nam KH, Paeng DG, Choi MJ, Shung KK. Ultrasonic observation of blood disturbance in a stenosed tube: Effects of flow acceleration and turbulence downstream. Ultrasound Med Biol 2008;34:114–122.
Nam KH, Paeng DG, Choi MJ. Ultrasonic backscatter from rat blood in aggregating media under in vitro rotational flow. IEEE Trans Ultrason Ferroelectr Freq Control 2009;56:270–279.
Nam KH, Yeom E, Ha H, Lee SJ. Simultaneous measurement of red blood cell aggregation and whole blood coagulation using high-frequency ultrasound. Ultrasound Med Biol 2012;38:468–475.
Nam KH, Bok TH, Kong Q, Paeng DG. High spatial and temporal resolution observations of pulsatile changes in blood echogenicity in the common carotid artery of rats. Ultrasound Med Biol 2013;39: 1665–1671.
Nemeth N, Kiss F, Klarik Z, Peto K, Vanyolos E, Toth L, Furka I, Miko I. Testicular ischemia–reperfusion may alter micro-rheological parameters in laboratory rats. Clin Hemorheol Microcirc 2014;57:243–253.
Nemeth N, Berhes M, Kiss F, Hajdu E, Deak A, Molnar A, Szabo J, Fulesdi B. Early hemorheological changes in a porcine model of intravenously given *E. coli* induced fulminant sepsis. Clin Hemorheol Microcirc 2015;61:479–496.
Neumann FJ, Katus HA, Hoberg E, Roebruck P, Braun M, Haupt HM, Tillmanns H, Kübler W. Increased plasma viscosity and erythrocyte aggregation: Indicators of an unfavourable clinical outcome in patients with unstable angina pectoris. Br Heart J 1991;66:425–430.

Nguyen LC, Yu FT, Cloutier G. Cyclic changes in blood echogenicity under pulsatile flow are frequency dependent. Ultrasound Med Biol 2008;34:664–673.

Nicolaides AN. From symptoms to leg edema: Efficacy of Daflon 500 mg. Angiology 2003;54:S33–S44.

Novacek G, Vogelsang H, Genser D, Moser G, Gangl A, Ehringer H, Koppensteiner R. Changes in blood rheology caused by Crohn's disease. Eur J Gastroenterol Hepatol 1996;8:1089–1093.

Oelze ML, Mamou J. Review of quantitative ultrasound: Envelope statistics and backscatter coefficient imaging and contributions to diagnostic ultrasound. IEEE Trans Ultrason Ferroelectr Freq Control 2016;63:336–351.

Paeng DG, Chiao RY, Shung KK. Echogenicity variations from porcine blood: I. The "bright collapsing ring" under pulsatile flow. Ultrasound Med Biol 2004;30:45–55.

Pecile P, Miorin E, Romanello C, Falleti E, Valent F, Giacomuzzi F, Tenore A. Procalcitonin: A marker of severity of acute pyelonephritis among children. Pediatrics 2004;114:e249–e254.

Peled N, Kassirer M, Kramer MR, Rogowski O, Shlomi D, Fox B, Berliner AS, Shitrit D. Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome. Thromb Res 2008; 121:631–636.

Potron G, Jolly D, Nguyen P, Mailliot JL, Pignon B. Approach to erythrocyte aggregation through erythrocyte sedimentation rate: Application of a statistical model in pathology. Nouv Rev Fr Hematol 1994;36: 241–247.

Pribush A, Meyerstein N. Methodological aspects of erythrocyte aggregation. Recent Pat Anticancer Drug Discov 2007;2:240–245.

Qin Z, Durand LG, Cloutier G. Kinetics of the "black hole" phenomenon in ultrasound backscattering measurements with red blood cell aggregation. Ultrasound Med Biol 1998;24:245–256.

Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, Gentleman S, Heckemann RA, Gunanayagam K, Gelosa G. Inflammation after trauma: Microglial activation and traumatic brain injury. Ann Neurol 2011;70:374–383.

Raz O, Rogowski O, Shapira I, Maharshak N, Karni Y, Berliner S. Dissociated effects of physical activity and weight loss on fibrinogen concentrations and markers of red blood cell aggregation: Relevance for life style modification in atherothrombosis. Clin Hemorheol Microcirc 2007;37:253–262.

Razavian SM, Guillemin MT, Guillet R, Beuzard Y, Boynard M. Assessment of red blood cell aggregation with dextran by ultrasonic interferometry. Biorheology 1991;28:89–97.

Razavian SM, Levenson J, Peronneau P, Simon A. Quantification of erythrocyte aggregation by blood echogenicity: A preliminary study. J Cardiovasc Surg (Torino) 1995;36:375–377.

Recchia D, Wickline SA. Ultrasonic tissue characterization of blood during stasis and thrombosis with a real-time linear-array backscatter imaging system. Coron Artery Dis 1993;4:987–994.

Reggiori G, Occhipinti G, De Gasperi A, Vincent JL, Piagnerelli M. Early alterations of red blood cell rheology in critically ill patients. Crit Care Med 2009;37:3041–3046.

Rogowski O, Zeltser D, Rotstein R, Shapira I, Avitzour D, Fusman R, Mardi T, Prochorov V, Arber N, Berliner S. Correlated expression of adhesive properties for both white and red blood cells during inflammation. Biorheology 2000;37:361–370.

Rogowski O, Berliner S, Zeltser D, Serov J, Ben-Assayag E, Justo D, Rozenblat M, Kessler A, Deutsch V Zakuth V. The erythrosense as a real-time biomarker to reveal the presence of enhanced red blood cell aggregability in atherothrombosis. Am J Ther 2005;12:286–292.

Rotstein R, Zeltser D, Shapira I, Berliner S, Avitzour D, Dwolatzky T, Arber N. An inflammation meter to reveal the presence and extent of inflammation in older patients. J Am Geriatr Soc 2000;48:1739–1741.

Rotstein R, Fusman R, Berliner S, Levartovsky D, Rogowsky O, Cohen S, Shabtai E, Shapira I, Avitzour D, Arber N, Zeltser D. The feasibility of estimating the erythrocyte sedimentation rate within a few minutes by using a simple slide test. Clin Lab Haematol 2001a;23: 21–25.

Rotstein R, Fusman R, Zeltser D, Shapira I, Shabtai E, Avitzour D, Sadees N, Levartovsky D, Arber N, Eldor A, Berliner S. The picture of inflammation: A new concept that combines the white blood cell count and erythrocyte sedimentation rate into a new hematologic diagnostic modality. Acta Haematol 2001b;106:106–114.

Rotstein R, Landau T, Twig A, Rubinstein A, Koffler M, Justo D, Constantiner D, Zeltser D, Shapira I, Mardi T. The erythrocyte adhesiveness/aggregation test (EAAT): A new biomarker to reveal the presence of low grade subclinical smoldering inflammation in individuals with atherosclerotic risk factors. Atherosclerosis 2002a; 165:343–351.

Rotstein R, Zeltser D, Shapira I, Avitzour D, Fusman R, Dvolatzki T, Loewenstein A, Aronson M, Bornstein N, Arber N, Berliner S. The usefulness of an inflammation meter to detect the presence of infection/inflammation in elderly patients. J Gerontol A Biol Sci Med Sci 2002b; 57:M122–M127.

Rouffiac V, Peronneau P, Hadengue A, Barbet A, Delouche P, Dantan P, Lassau N, Levenson J. A new ultrasound principle for characterizing erythrocyte aggregation: In vitro reproducibility and validation. Invest Radiol 2002;37:413–420.

Rouffiac V, Peronneau P, Guglielmi JP, Del-Pino M, Lassau N, Levenson J. Comparison of new ultrasound index with laser reference and viscosity indexes for erythrocyte aggregation quantification. Ultrasound Med Biol 2003;29:789–799.

Rouffiac V, Guglielmi JP, Barbet A, Lassau N, Peronneau P. Application of validated ultrasound indices to investigate erythrocyte aggregation in pigs: Preliminary in vivo results. Ultrasound Med Biol 2004;30:35–44.

Saha RK, Kolios MC. A simulation study on photoacoustic signals from red blood cells. J Acoust Soc Am 2011;129:2935–2943.

Samocha-Bonet D, Lichtenberg D, Tomer A, Deutsch V, Mardi T, Goldin Y, Abu-Abeid S, Shenkerman G, Patshornik H, Shapira I, Berliner S. Enhanced erythrocyte adhesiveness/aggregation in obesity corresponds to low-grade inflammation. Obes Res 2003;11:403–407.

Samocha-Bonet D, Ami RB, Shapira I, Shenkerman G, Abu-Abeid S, Stern N, Mardi T, Tulchinski T, Deutsch V, Yedgar S. Flow-resistant red blood cell aggregation in morbid obesity. Int J Obes Relat Metab Disord 2004;28:1528–1534.

Sandor B, Nagy A, Toth A, Rabai M, Mezey B, Csatho A, Czuriga I, Toth K, Szabados E. Effects of moderate aerobic exercise training on hemorheological and laboratory parameters in ischemic heart disease patients. PLoS ONE 2014;9:e110751.

Saniabadi AR, Lowe GD, Madhok R, Spowart K, Shaw B, Barbenel JC, Forbes CD. Red blood cells mediate spontaneous aggregation of platelets in whole blood. Atherosclerosis 1987;66:175–180.

Santos MJ, Pedro LM, Canhão H, e Fernandes JF, da Silva JC, Fonseca JE, Saldanha C. Hemorheological parameters are related to subclinical atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis patients. Atherosclerosis 2011;219:821–826.

Sargento L, Saldanha C, Monteiro J, Perdigão C, Silva JM. Evidence of prolonged disturbances in the haemostatic, hemorheologic and inflammatory profiles in transmural myocardial infarction survivors: A 12-month follow-up study. Thromb Haemost 2003;89:892–903.

Sargento L, Saldanha C, Monteiro J, Perdigão C, e Silva JM. Long-term prognostic value of protein C activity, erythrocyte aggregation and membrane fluidity in transmural myocardial. Thromb Haemost 2005;94:380–388.

Sato T, Watanabe Y. High sensitivity estimation of red blood cell aggregation with ultrasonic peak frequency. Proc IEEE Int Ultrason Symp 2013;868–871.

Schechner V, Shapira I, Berliner S, Comaneshter D, Hershcovici T, Orlin J, Zeltser D, Rozenblat M, Lachmi K, Hirsch M. Significant dominance of fibrinogen over immunoglobulins, C-reactive protein, cholesterol and triglycerides in maintaining increased red blood cell adhesiveness/aggregation in the peripheral venous blood: A model in hypercholesterolaemic patients. Eur J Clin Invest 2003;33:955–961.

Schiessl B. Inflammatory response in preeclampsia. Mol Aspects Med 2007;28:210–219.

Sennaoui A, Boynard M, Pautou C. Characterization of red blood cell aggregate formation using an analytical model of the ultrasonic backscattering coefficient. IEEE Trans Biomed Eng 1997;44:585–591.

Sharshun Y, Brill S, Mardi T, Justo D, Rozenblat M, Goldin Y, Serov J, Berliner S, Shapira I. Inflammation at a glance: Erythrocyte adhesiveness/aggregation test to reveal the presence of inflammation in people with atherothrombosis. Heart Dis 2003;5:182–183.

Shehada RE, Cobbold RS, Mo LY. Aggregation effects in whole blood: Influence of time and shear rate measured using ultrasound. Biorheology 1994;31:115–135.

Shenhar-Tsarfaty S, Assayag EB, Bova I, Shopin L, Berliner S, Shapira I, Bornstein NM. Early signaling of inflammation in acute ischemic stroke: Clinical and rheological implications. Thromb Res 2008; 122:167–173.

Shin S, Yang Y, Suh JS. Measurement of erythrocyte aggregation in a microchip stirring system by light transmission. Clin Hemorheol Microcirc 2009;41:197–207.

Shung KK, Paeng DG. Ultrasound: An unexplored tool for blood flow visualization and hemodynamic measurements. Jpn J Appl Phys 2003; 42:2901.

Shung KK, Sigelmann RA, Reid JM. Scattering of ultrasound by blood. IEEE Trans Biomed Eng 1976;460–467.

Sigel B, Coelho JC, Schade SG, Justin J, Spigos DG. Effect of plasma proteins and temperature on echogenicity of blood. Invest Radiol 1982; 17:29–33.

Sigel B, Machi J, Beitler JC, Justin JR. Red cell aggregation as a cause of blood-flow echogenicity. Radiology 1983;148:799–802.

Sigel B, Machi J, Beitler JC, Ramos JR, Justin JR, Feinberg H. Ultrasonic detection of red cell aggregation immediately preceding blood clotting. Invest Radiol 1984;19:458–461.

Simmonds MJ, Sabapathy S, Serre KR, Haseler LJ, Gass GC, Marshall-Gradisnik SM, Minahan CL. Regular walking improves plasma protein concentrations that promote blood hyperviscosity in women 65–74 yr with type 2 diabetes. Clin Hemorheol Microcirc 2016;64:189–198.

Spengler M, Svetaz M, Leroux M, Bertoluzzo S, Carrara P, Van Isseldyk F, Petrelli D, Parente F, Bosch P. Erythrocyte aggregation in patients with systemic lupus erythematosus. Clin Hemorheol Microcirc 2011;47:279–285.

Steinvil A, Arbel Y, Leshem-Rubinow E, Halkin A, Keren G, Revivo M, Finkelstein A, Cohen M, Tal R, Aviram G, Berliner S, Banai S. Erythrocyte aggregation portends worse outcomes in unstable angina patients undergoing percutaneous coronary interventions. Clin Hemorheol Microcirc 2013;55:213–221.

Stoltz JF, Gaillard S, Paulus F, Henri O, Dixneuf P. Experimental approach to rouleau formation. Comparison of three methods. Biorheology Suppl 1984;1:221–226.

Sugata Y, Ito M. A preliminary study on quantification of spontaneous echo contrast in superficial vein. Acta Clin Croat 2012;51(1 Suppl): 141–147.

Szentkereszty Z, Kotan R, Kiss F, Klarik Z, Posan J, Furka I, Sapy P, Miko I, Peto K, Nemeth N. Effects of various drugs (flunixin, pentoxifylline, enoxaparin) modulating micro-rheological changes in cerulein-induced acute pancreatitis in the rat. Clin Hemorheol Microcirc 2014;57:303–314.

Thavasu P, Longhurst S, Joel S, Slevin M, Balkwill F. Measuring cytokine levels in blood: Importance of anticoagulants, processing, and storage conditions. J Immunol Methods 1992;153:115–124.

Toker S, Rogowski O, Melamed S, Shirom A, Shapira I, Berliner S, Zeltser D. Association of components of the metabolic syndrome with the appearance of aggregated red blood cells in the peripheral blood: An unfavorable hemorheological finding. Diabetes Metab Res Rev 2005; 21:197–202.

Totsimon K, Biro K, Szabo ZE, Toth K, Kenyeres P, Marton Z. The relationship between hemorheological parameters and mortality in critically ill patients with and without sepsis. Clin Hemorheol Microcirc 2017;65:11–29.

Tripette J, Denault AY, Allard L, Chayer B, Perrault LP, Cloutier G. Ultrasound monitoring of RBC aggregation as a real-time marker of the inflammatory response in a cardiopulmonary bypass swine model. Crit Care Med 2013;41:e171–e178.

Tripette J, Nguyen LC, Allard L, Robillard P, Soulez G, Cloutier G. In vivo venous assessment of red blood cell aggregate sizes in diabetic patients with a quantitative cellular ultrasound imaging method: Proof of concept. PLoS ONE 2015;10:e0124712.

Urbach J, Rotstein R, Fusman R, Zeltser D, Shapira I, Branski D, Berliner S. Reduced acute phase response to differentiate between viral and bacterial infections in children. Pediatr Pathol Mol Med 2002;21: 557–567.

Urbach J, Elishkevitz K, Rotstein R, Rozenblat MV, Mardi T, Shapira I, Brandski D, Berline S. Telemedicine-based application for the detection of inflammation in pediatrics. Telemed J E Health 2003;9: 241–245.

Urbach J, Shapira I, Branski D, Berliner S. Acute phase response in the diagnosis of bacterial infections in children. Pediatr Infect Dis J 2004; 23:159–160.

Urbach J, Rogowski O, Shapira I, Avitzour D, Branski D, Schwartz S, Berliner S, Mardi T. Automatic 3-dimensional visualization of peripheral blood slides: A new approach for the detection of infection/inflammation at the point of care. Arch Pathol Lab Med 2005;129: 645–650.

Urdulashvili T, Momtselidze N, Mantskava M, Narsia N, McHedlishvili G. Hemorheological, microvascular and hemodynamic disorders during coronary heart disease. Georgian Med News 2006;136:55–57.

van Rooy MJ, Pretorius E. Platelet interaction with erythrocytes and propensity to aggregation in essential thrombocythaemia. Lancet 2016; 387:1210.

Valles J, Santos MT, Aznar J, Martinez M, Moscardo A, Pinon M, Broekman MJ, Marcus AJ. Platelet-erythrocyte interactions enhance alpha(IIb)beta(3) integrin receptor activation and P-selectin expression during platelet recruitment: Down-regulation by aspirin ex vivo. Blood 2002;99:3978–3984.

Vayá A, Martinez M, Dalmau J. Hemorheological changes in children with familial hypercholesterolemia. Clin Hemorheol 1996;16:549–557.

Vayá A, Todolí J, Calvo J, Romagnoli M, Ricart JM. Haemorheological profile in patients with systemic sclerosis. Clin Hemorheol Microcirc 2008;40:243–248.

Vayá A, Hernández-Mijares A, Suescun M, Solá E, Cámara R, Romagnoli M, Bautista D, Laiz B. Metabolic alterations in morbid obesity: Influence on the haemorheological profile. Clin Hemorheol Microcirc 2011a;48:247–255.

Vayá A, Hernandez-Mijares A, Bonet E, Sendra R, Sola E, Perez R, Corella D, Laiz B. Association between hemorheological alterations and metabolic syndrome. Clin Hemorheol Microcirc 2011b; 49:493–503.

Vayá A, Alis R, Romagnoli M, Perez R, Bautista D, Alonso R, Laiz B. Rheological blood behavior is not only influenced by cardiovascular risk factors but also by aging itself: Research into 927 healthy Spanish Mediterranean subjects. Clin Hemorheol Microcirc 2013a; 54:287–296.

Vayá A, Alis R, Hernandez JL, Calvo J, Mico L, Romagnoli M, Ricarte JM. RDW in patients with systemic lupus erythematosus: Influence of anaemia and inflammatory markers. Clin Hemorheol Microcirc 2013b;54:333–339.

Vayá A, Ricart JM, Andino B, Todoli J, Nuñez C, Calvo J, Bautista D. Psoriasis and hemorheology. Influence of the metabolic syndrome. Clin Hemorheol Microcirc 2013c;55:331–339.

Volz KR, Evans KD, Kanner CD, Basso DM. Exploring targeted contrast-enhanced ultrasound to detect neural inflammation: An example of standard nomenclature. J Diagn Med Sonogr 2016;32:313–323.

Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: Mechanisms involved and possible therapeutic strategies. Chest 1997;112:676–692.

Wang JS, Fu TC, Wang CH, Chou SL, Liu MH, Cherng WJ. Exertional periodic breathing potentiates erythrocyte rheological dysfunction by elevating pro-inflammatory status in patients with anemic heart failure. Int J Cardiol 2013;167:1289–1297.

Wang SH, Shung KK. In vivo measurements of ultrasonic backscattering in blood. IEEE Trans Ultrason Ferroelectr Freq Control 2001; 48:425–431.

Wang XF, Liu L, Cheng TO, Li ZA, Deng YB, Wang JE. The relationship between intracardiovascular smoke-like echo and erythrocyte rouleaux formation. Am Heart J 1992;124:961–965.

Warltier DC, Laffey JG, Boylan JF, Cheng DC. The Systemic Inflammatory Response to Cardiac SurgeryImplications for the Anesthesiologist. J Am Soc Anesthesiol 2002;97:215–252.
Watts T, Barigou M, Nash GB. Comparative rheology of the adhesion of platelets and leukocytes from flowing blood: Why are platelets so small? Am J Physiol Heart Circ Physiol 2013;304:H1483–H1494.
Weng X, Cloutier G, Beaulieu R, Roederer GO. Influence of acute-phase proteins on erythrocyte aggregation. Am J Physiol Heart Circ Physiol 1996;271:H2346–H2352.
Wiewiora M, Piecuch J, Gluck M, Slowinska-Lozynska L, Sosada K. Shear stress and flow dynamics of the femoral vein among obese patients who qualify for bariatric surgery. Clin Hemorheol Microcirc 2013;54:313–323.
Woodward M, Rumley A, Lowe GD, Tunstall-Pedoe H. C-reactive protein: Associations with haematological variables, cardiovascular risk factors and prevalent cardiovascular disease. Br J Haematol 2003;122:135–141.
Xu X, Yu L, Chen Z. Velocity variation assessment of red blood cell aggregation with spectral domain Doppler optical coherence tomography. Ann Biomed Eng 2010;38:3210–3217.
Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokayama T, Tsukaguchi K, Narita N. Airway Inflammation in COPD Assessed by sputum levels of interleukin-8. Chest 1997;112:505–510.
Yedgar S, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. Pathophysiol Haemost Thromb 2002;32:263–268.
Yeom E, Lee SJ. Microfluidic-based speckle analysis for sensitive measurement of erythrocyte aggregation: A comparison of four methods for detection of elevated erythrocyte aggregation in diabetic rat blood. Biomicrofluidics 2015a;9:024110, 1-15.
Yeom E, Lee SJ. Relationship between velocity profile and ultrasound echogenicity in pulsatile blood flows. Clin Hemorheol Microcirc 2015b;59:197–209.
Yeom E, Nam KH, Paeng DG, Lee SJ. Effects of red blood cell aggregates dissociation on the estimation of ultrasound speckle image velocimetry. Ultrasonics 2014;54:1480–1487.
Yeom E, Jun Kang Y, Lee SJ. Hybrid system for ex vivo hemorheological and hemodynamic analysis: A feasibility study. Sci Rep 2015;5:11064.
Yeom E, Kim HM, Park JH, Choi W, Doh J, Lee SJ. Microfluidic system for monitoring temporal variations of hemorheological properties and platelet adhesion in LPS-injected rats. Sci Rep 2017;7:1801.
Yu FT, Cloutier G. Experimental ultrasound characterization of red blood cell aggregation using the structure factor size estimator. J Acoust Soc Am 2007;122:645–656.
Yu FT, Franceschini E, Chayer B, Armstrong JK, Meiselman HJ, Cloutier G. Ultrasonic parametric imaging of erythrocyte aggregation using the structure factor size estimator. Biorheology 2009;46:343–363.
Yu FT, Armstrong JK, Tripette J, Meiselman HJ, Cloutier G. A local increase in red blood cell aggregation can trigger deep vein thrombosis: Evidence based on quantitative cellular ultrasound imaging. J Thromb Haemost 2011;9:481–488.
Yuan YW, Shung KK. Ultrasonic backscatter from flowing whole blood: I. Dependence on shear rate and hematocrit. J Acoust Soc Am 1988a;84:52–58.
Yuan YW, Shung KK. Ultrasonic backscatter from flowing whole blood: II. Dependence on frequency and fibrinogen concentration. J Acoust Soc Am 1988b;84:1195–1200.
Yuan YW, Shung KK. Echoicity of whole blood. J Ultrasound Med 1989;8:425–434.
Zakynthinos E, Pappa N. Inflammatory biomarkers in coronary artery disease. J Cardiol 2009;53:317–333.
Zannad F, Voisin P, Brunotte F, Bruntz JF, Stoltz JF, Gilgenkrantz JM. Haemorheological abnormalities in arterial hypertension and their relation to cardiac hypertrophy. J Hypertens 1988;6:293–297.
Zeltser D, Rogowski O, Berliner S, Mardi T, Justo D, Serov J, Rozenblat M, Avitzour D, Shapira I. Sex differences in the expression of haemorheological determinants in individuals with atherothrombotic risk factors and in apparently healthy people. Heart 2004a;90:277–281.
Zeltser D, Serov J, Mardi T, Rogowski O, Tulshinski T, Goldin Y, Justo D, Aharonov S, Rozenblat M, Berliner S. Serum lipids as minor determinants of the degree of erythrocyte adhesiveness/aggregation in the peripheral blood of individuals with low grade inflammation and moderately increased serum lipids. Clin Hemorheol Microcirc 2004b;31:161–167.
Zhou X, Fragala MS, McElhaney JE, Kuchel GA. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. Curr Opin Clin Nutr Metab Care 2010;13:541–547.
Zilberman L, Rogowski O, Rozenblat M, Shapira I, Serov J, Halpern P, Dotan I, Arber N, Berliner S. Inflammation-related erythrocyte aggregation in patients with inflammatory bowel disease. Dig Dis Sci 2005;50:677–683.
Zimran A, Bashkin A, Elstein D, Rudensky B, Rotstein R, Rozenblat M, Mardi T, Zeltser D, Deutsch V, Shapira I. Rheological determinants in patients with Gaucher disease and internal inflammation. Am J Hematol 2004;75:190–194.

APPENDIX. DETAILS OF SEARCH STRATEGY

First conceptual group in Pubmed: ("Erythrocyte Aggregation" [Mesh] OR "Erythrocyte Deformability" [Mesh] OR erythrocyte rouleaux formation [Title/Abstract] OR erythrocyte rouleaux formation [Other Term] OR erythrocyte aggregation [Title/Abstract] OR erythrocyte aggregation [Other Term] OR erythrocytes aggregation [Title/Abstract] OR erythrocytes aggregation [Other Term]

OR

(red blood cell* [Title/Abstract] OR red blood cell* [Other Term] OR red cell* [Title/Abstract] OR red cell* [Other Term] OR RBCs [Title/Abstract] OR RBCs [Other Term] OR erythrocyte* [Title/Abstract] OR erythrocyte* [Other Term]

AND

Deformabilit* [Title/Abstract] OR deformabilit* [Other Term] OR aggregation* [Title/Abstract] OR aggregation* [Other Term])

AND

Second conceptual group in Pubmed: ("Inflammation" [Mesh] OR "Inflammation Mediators" [Mesh] OR inflammat* [Title/Abstract] OR inflammat* [Other Term])

OR

("Ultrasonography" [Mesh] OR "Diagnostic Imaging" [Mesh] OR "Ultrasonography, Doppler, Color" [Mesh] OR "Ultrasonography, Doppler, Pulsed" [Mesh] OR "Ultrasonography, Doppler, Duplex" [Mesh] OR "Ultrasonography, Doppler" [Mesh] OR "Ultrasonics" [Mesh] OR ultrasonograph* [Title/Abstract] OR ultrasonograph* [Other Term] OR diagnostic imaging [Title/Abstract] OR diagnostic imaging [Other Term] OR ultrasound* [Other Term] OR ultrasound* [Title/Abstract] OR photoacoustic* [Title/Abstract] OR photoacoustic* [Other Term] OR "Photoacoustic Techniques" [Mesh] OR echograph* [Title/Abstract] OR echograph* [Other Term])

Both conceptual groups were translated and adapted for each database. No filter by language, date of publication or type of publication was used. Hand searching was also used to identify other references. We removed duplicates with EndNote.