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Point-of-care coagulation monitoring: first clinical experience using a paper-based lateral flow diagnostic device

Michael A. Hegener[1] · Hua Li[2] · Daewoo Han[2] · Andrew J. Steckl[2] ·
Giovanni M. Pauletti[1]

Abstract Vitamin K antagonists such as warfarin are the most widely used class of oral anticoagulants. Due to a narrow therapeutic window, patients on warfarin require regular monitoring. Self-testing using point-of-care (POC) diagnostic devices is available, but cost makes this monitoring method beyond reach for many. The main objective of this research was to assess the clinical utility of a low-cost, paper-based lateral flow POC diagnostic device developed for anticoagulation monitoring without the need for a separate electronic reader. Custom-fabricated lateral flow assay (LFA) test strips comprised of a glass fiber sample pad, a nitrocellulose analytical membrane, a cellulose wicking pad, and a plastic backing card were assembled in a plastic cassette. Healthy volunteers and patients on warfarin therapy were recruited for this prospective study. For each participant, a whole blood sample was collected via fingerstick to determine: (1) international normalized ratio (INR) using the CoaguChek® XS coagulometer, (2) hematocrit by centrifugation, and (3) red blood cell (RBC) travel distance on the experimental LFA device after 240 s using digital image analysis. RBC travel distance measured on the LFA device using blood samples obtained from warfarin patients positively correlated with increasing INR value and the LFA device had the capability to statistically distinguish between healthy volunteer INR values and those for patients groups with INR $\geq$ 2.6. From these data, it is predicted that this low-cost, paper-based LFA device can have clinical utility for identifying anticoagulated patients taking vitamin K antagonists who are outside of the desired therapeutic efficacy window.

1 Introduction

Thrombosis is a leading cause of morbidity and mortality worldwide by representing the underlining pathology for myocardial infarction, venous thromboembolism, and thromboembolic stroke (Lozano et al. 2012). Clinical guidelines give preference to outpatient treatment of uncomplicated thromboembolic events, such as deep vein thrombosis and pulmonary embolism, with oral anticoagulants being the predominate means of therapy (Kearon et al. 2016). In addition, oral anticoagulants are widely utilized for primary and secondary prevention of thromboembolic stroke in patients with atrial fibrillation (You et al. 2012). Despite the recent approval of several novel oral anticoagulants such as dabigatran, rivaroxaban, apixiban, and edoxaban that do not require strict therapeutic monitoring, vitamin K antagonists such as warfarin, acenocoumarol, and phenprocoumon remain the most widely used class of oral anticoagulants, especially in low-income/developing countries and for selected patient populations, including those with impaired kidney and liver function (Onundarson et al. 2015). It is also predicted that physicians will continue prescribing warfarin because of the ability to reverse the anticoagulant effect using vitamin K. To date, the only novel oral anticoagulant with an FDA-approved reversal agent is dabigatran (Eikelboom et al. 2015). Unfortunately,

Michael A. Hegener and Hua Li contributed equally to this work

✉ Michael A. Hegener
michael.hegener@uc.edu

[1] James L. Winkle College of Pharmacy, University of Cincinnati, 3225 Eden Avenue, Cincinnati, OH 45267, USA

[2] Nanoelectronics Laboratory, Department of Electrical Engineering and Computer Science, University of Cincinnati, Cincinnati, OH 45221, USA

therapeutic anticoagulation using oral vitamin K antagonists is associated with large inter-individual variability in dose response. Due to a narrow therapeutic window, anticoagulated patients on warfarin or other vitamin K antagonists require regular monitoring (Ansell et al. 2008). Lack of patient access to adequate monitoring, however, carries an increased risk of thrombotic and hemorrhagic complications and may be a deterrent to their use. It is estimated, that anticoagulant therapy is underutilized in approximately half of the patient population diagnosed with atrial fibrillation and risk factors for stroke (Ogilvie et al. 2010, Pugh et al. 2011, Hess et al. 2014).

Inhibition of vitamin K reduces γ-carboxylation of clotting factors II, VII, IX, and X, which is normally required to initiate blood clot formation. Subsequent changes in prothrombin time can be quantified using various *in vitro* assays. To adjust for inter-laboratory variability in prothrombin time determination, the international normalized ratio (INR) was defined in 1983 and has represented for decades the "gold standard" for routine monitoring of the pharmacodynamics efficacy of vitamin K antagonists. The desired INR range for most indications is 2.0 to 3.0 (Coumadin® 2017). Traditionally, blood coagulation testing is performed by the primary care provider using a blood draw by venipuncture and subsequent laboratory analysis. Alternatively, patients are able to access specialized anticoagulation clinics that employ point-of-care (POC) INR measurements using a fingerstick blood draw followed by therapeutic management by a health care provider. In many developed countries, however, patients are now given the opportunity for self-testing using portable POC devices. The results from these measurements will be communicated to a health care provider who can recommend dose adjustments (O'Shea et al. 2008).

The main impetus for the development of POC testing devices is cost reduction and improved monitoring capabilities. Evidence-based research underlines that outpatient anticoagulation management translates into significant patient benefits, including longer time within the desired therapeutic range of vitamin K antagonists and lower incidence of thromboembolic events when compared to usual care (Chiquette et al. 1998). Similarly, patients or their caregivers have embraced self-testing using a POC testing device as it offers substantially greater convenience while reducing thromboembolic events without an increased risk for serious bleeding when compared with usual care (Bloomfield et al. 2011). In the United States, the mean cost associated with INR monitoring across all testing methodologies is $69.76 per sample, with anticoagulation clinics costing more than usual care (Anderson et al. 2015). The price of marketed POC anticoagulation monitoring devices ranges between $600–700 for the reader and $5–6 per test strip, respectively (Li et al. 2017). Unless reimbursed by an insurance company, these costs render POC anticoagulation testing beyond reach for a significant segment of the population in developed

countries and certainly unaffordable for patients in low-income/developing countries (CAP Today 2017).

As life expectancy in most developed and developing countries is gradually rising due to improved social and health policies, health care systems in developing countries are significantly more challenged by a simultaneous burden of disease that involves lifestyle diseases such as stroke and atrial fibrillation in parallel to communicable diseases such as HIV/AIDS, malaria, or tuberculosis, which are persistent in the developing world (Stambler and Ngunga 2015). Consequently, global demand for cost-effective anticoagulant therapy using vitamin K antagonists remains high. To enable adequate management of those patients, however, it will be imperative to develop new low-cost diagnostic tools that allow blood coagulation monitoring without the requirement of an expensive reader and at a much lower price per test than that of existing products as defined by the ASSURED guidelines established by the World Health Organization (Hawkins and Weigl 2010).

This manuscript summarizes the first clinical results obtained with an experimental, paper-based microfluidic lateral flow assay (LFA) device engineered for monitoring of blood coagulation. Consistent with conventional LFA technology, this novel paper-based diagnostic device is comprised of a sample pad, a porous membrane, and a wicking pad (Fig. 1). It is a self-powered device that does not require an external power source and, due to its simplicity in design, has very low material costs. Once whole blood is applied to the sample pad, it migrates onto the porous membrane where separation of red blood cells (RBCs) from plasma occurs. Since the viscosity of whole blood changes with its coagulation ability, the distance traveled by RBCs in a given time is related to its coagulation
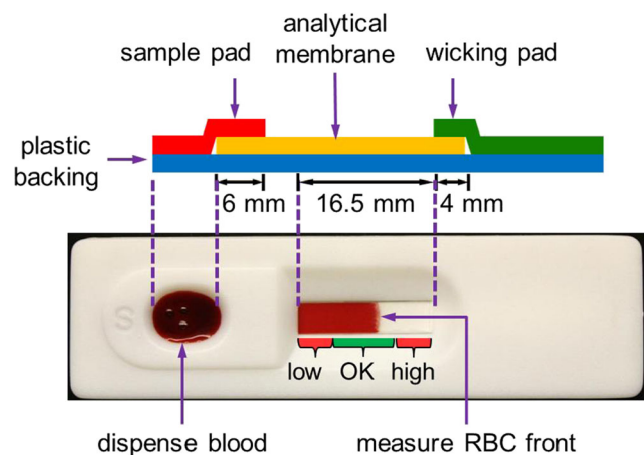

Fig. 1 Schematic design and photograph of the experimental, paper-based microfluidic lateral flow assay (LFA) device engineered for qualitative monitoring of blood coagulation without the need of an additional reader. It is hypothesized that travel distance of RBCs on this experimental LFA device correlates with clinical efficacy of anticoagulants allowing qualitative assessment of blood coagulation status using the unaided eye

state (Ranucci et al. 2014). The RBC travel distance, which is readily visible to the unaided eye, serves as endpoint marker.

2 Materials and methods
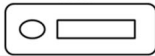
2.1 Device fabrication

The LFA test strip evaluated in this clinical feasibility study was assembled using a glass fiber sample pad (Ahlstrom, Alpharetta, GA), a nitrocellulose analytical membrane (Millipore, Billerica, MA), a cellulose wicking pad (Whatman, Little Chalfont, United Kingdom), and a plastic backing card (Diagnostic Consulting Network, Carlsbad, CA). The overall strip dimensions were 4 mm × 53 mm containing a 13 mm long sample pad, a 30 mm analytical membrane, and 20 mm long wicking pad. The three components were stacked on an opaque plastic backing card that was, subsequently, cut into strips of 4 mm width using a guillotine paper trimmer. Each fabricated LFA test strip was secured inside a plastic cassette (Diagnostic Consulting Network, Carlsbad, CA) consisting of two pieces that snap together. The assembled test kit had a sample dispense window and a 16.5 mm long observation window (see Fig. 1). The observation window was covered with a transparent adhesive tape to minimize evaporation of sample fluid. LFA devices were sealed individually in air-tight clear plastic pouches, each containing a 1 g silica gel desiccant packet. Diagnostic devices from one fabrication batch were stored at a controlled room temperature (20–22 °C) and used for clinical assessment

within 3 months of production. The bill of materials for a single LFA device used in this clinical feasibility study was $0.38 (Fig. 2). It is estimated that material cost can be reduced to $0.24/test kit when producing in large quantities (>50,000 units).

2.2 Study setting and population

This prospective clinical feasibility study was performed at two independent sites, including both healthy, non-anticoagulated individuals and patients on prescribed oral warfarin dosing regimens. Study participants taking prescribed oral warfarin were recruited at the St. Elizabeth Healthcare anticoagulation clinic (Fort Thomas, KY). Inclusion of subjects in this warfarin patient study arm was limited to established patients at the St. Elizabeth clinic who have been on warfarin therapy for at least 1 month, were at least 18 years of age, and mentally competent. Patients with diagnosed blood clotting disorders such as factor V Leiden, antiphospholipid syndrome, protein C deficiency, protein S deficiency, or antithrombin deficiency, as well as pregnant women, were excluded from this study. Healthy volunteers were recruited from the student, faculty, and staff population at the University of Cincinnati (Cincinnati, OH). Participants were eligible if at least 18 years of age and mentally competent. Exclusion criteria included prior history of any anticoagulant therapy in the previous 3 months, the presence of any known blood clotting disorders, and pregnancy. This study received IRB approval from the University of Cincinnati as well as St. Elizabeth Healthcare.

Fig. 2 Bill of materials for fabrication of experimental LFA device

LFA Component	Cost (USD)
53 mm × 300 mm Plastic Backing Card [a] (Diagnostic Consulting Network, MIBA-020)	1.20
13 mm × 300 mm Glass Fiber Sample Pad [a] (Ahlstrom 8950)	0.12
30 mm × 300 mm Nitrocelulose Membrane [a] (Millipore HF075)	1.61
20 mm × 300 mm Cotton Fiber Wicking Pad [a] (Whatman 470)	0.24
Single LFA Strip	(1.20+0.12+1.61+0.24)/40=0.08
Cassette (Diagnostic Consulting Network, MICA-125)	0.30
Single LFA Device	0.08+0.30=0.38

[a] materials needed to make 40 LFA strips

2.3 Study protocol

After written informed consent was obtained from an eligible study participant, the following tests were performed using a whole blood sample collected via fingerstick: (1) PT/INR using the CoaguChek® XS point-of-care coagulometer (Roche Diagnostics, Mannheim, Germany), (2) hematocrit measured after centrifugation of whole capillary blood, and (3) RBC travel distance on the experimental LFA device after 240 s. All experiments were performed by the primary investigator, but results were scored by a blinded secondary investigator. The principle of PT/INR measurements using the CoaguChek® XS has been described elsewhere (CoaguChek XS PT Test 2016). Briefly, a fingertip of the study participant was cleaned with an alcohol swab before a fingerstick was performed using a 1.8 mm, 23 gauge CoaguChek Lancet (Roche, Mannheim, Germany). Approximately 8–10 μL of whole capillary blood was aspirated using a CoaguChek capillary blood collection tube and applied within 10 s to a commercial CoaguChek XS test strip. Subsequently, 30 μL of whole capillary blood was obtained from the same fingerstick site using a calibrated Microsafe® pipette (Safe-Tec Clinical Products, Warminster, PA) and applied within 10 s to the sample pad of the experimental LFA device. If this second sample was not obtainable within 10 s of initial puncture, a second fingerstick on a different fingertip was performed to collect this additional blood sample. For hematocrit determination, 30 μL of whole capillary blood was obtained from a separate fingerstick performed on a different finger using a heparinized capillary tube (Drummond Scientific Company, Broomall, PA). Capillary tubes were placed into a Zipocrit® centrifuge (LW Scientific, Lawrenceville, GA) and spun for 5 min at 11,000 rpm. Hematocrit value was determined visually using a standard nomogram. The RBC travel distance on the experimental LFA device was measured using digital high resolution images that were acquired every 15 s during the entire test run of 4 min using a Canon EOS T4i digital camera. Image files were reviewed by a blinded secondary investigator who determined the travel distance at 240 s in millimeters using the ImageJ software (Schneider et al. 2012). The furthest point of the rounded RBC front was selected consistently for quantifying the RBC travel distance on the experimental LFA test strip.

2.4 Statistical analysis

Results are reported as mean ± standard deviation (SD). Statistically significant differences ($p < 0.05$) between groups were evaluated by employing one-way analysis of variance (ANOVA) using the Dunnett's multiple comparisons test (GraphPad Prism 7, GraphPad Software, La Jolla, CA).

3 Results

To explore the relationship between dynamic viscosity of an aqueous solution and the speed of movement of a solution sample on the porous substrate of the fabricated LFA device, the travel time required for different glycerol/water mixtures to reach the end of the observation window (i.e., 16.5 mm) was quantified. Glycerol and water were mixed at different weight ratios affording aqueous solutions with dynamic viscosity values between 2 and 21 cP (Segur and Oberstar 1951). The results of this validation experiment are shown in Fig. 3. The linear relationship between these parameters as determined by statistical regression analysis underlines that samples exhibiting lower viscosity travel proportionally faster on the porous membrane than samples of greater viscosity. Consequently, it was concluded that coagulation-induced changes in viscosity of a blood sample will lead to a distinct travel distance of RBCs within a predetermined observation or "assay" time. Furthermore, it was hypothesized that the RBC travel distance of blood samples exhibiting similar coagulation properties (i.e., similar INR values) is within a narrow range of the observation window.

For the initial clinical evaluation, 25 healthy volunteers were recruited using an IRB-approved protocol. The participant demographics summarized in Table 1 identify this cohort as predominantly female, between 21 and 32 years of age, and racially diverse. To correlate the results obtained using the experimental LFA device with quantitative INR values measured using a marketed POC diagnostic device, capillary blood samples collected from the each individual after a fingerstick were analyzed in parallel on the CoaguChek® XS and the LFA device. Figure 4a depicts the setup that was used to photographically capture the RBC travel distance on the LFA device. Quantitative endpoint analysis was
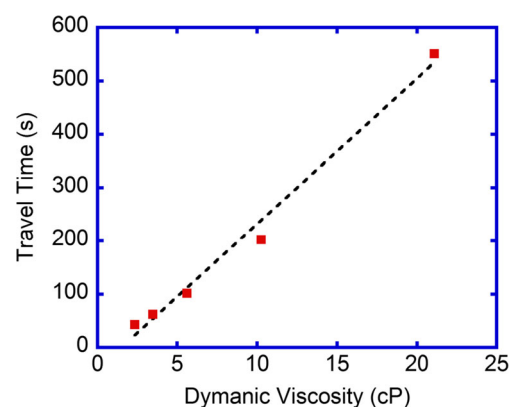

Fig. 3 Correlation of dynamic viscosity of aqueous glycerol solutions with flow properties measured on experimental LFA device. Travel time required for the solvent front to reach the end of the observation window on the experimental LFA device at room temperature was measured using high resolution digital photographs. Measurements were performed in duplicate. Dashed line represents liner regression line calculated for the entire data set

Table 1 Demographics of healthy volunteers and anticoagulated warfarin patients enrolled in clinical assessment of experimental LFA device for blood coagulation monitoring

Characteristic	Healthy Volunteers (n = 25)	Warfarin Patients (n = 28)
Age mean ± SD	26.7 ± 5.2	69.9 ± 11.9
Sex (% male)	36.0	28.6
Race		
Caucasian	60%	100%
Asian	40%	0%
Hematocrit [a] mean ± SD, (Min, Max)	42.5 ± 2.8 (39.0, 48.0)	40.6 ± 4.9 (30.0, 50.0)
INR [b] mean ± SD, (Min, Max)	1.0 ± 0.1 (0.9, 1.1)	2.6 ± 0.6 (1.6, 3.8)

[a] determined in sealed capillaries by conventional centrifugation

[b] quantified using the commercial CoaguChek® XS device

unambiguously performed by digital image analysis as outlined in Materials and Methods. Consistent with the expectations for healthy volunteers, INR determinations using the CoaguChek® XS demonstrate physiological blood coagulation properties for all individuals in this control group without outliers (INR = 0.9 to 1.1, Table 1). However, direct correlation between INR and corresponding RBC travel distance reveals substantial variability in the movement of coagulating blood on the fabricated LFA device despite a consistent INR value. This is illustrated in Fig. 5a where the RBC travel distance determined for 19 healthy volunteers with INR = 1.0 ranges from 4.15 to 10.80 mm at the end of the 240 s assay time. Similarly, the RBC front measured for 5 healthy volunteers with INR = 1.1 varies between 6.18 and 9.65 mm after the same time frame. To delineate whether the experimental variability in RBC travel distance observed for health volunteers is associated with insufficient precision of this paper-based diagnostic test or the result of a confounding variable affecting RBC travel distance on the LFA device, experimental data were stratified into three different groups using the hematocrit measured for each individual subject. Statistical comparison between these groups reveals significantly slower RBC movement on the experimental LFA device when blood samples contain a greater fraction of RBCs

(i.e., greater hematocrit value, Fig. 5b). Stratified data analysis within the healthy volunteer group using hematocrit as selection factor demonstrates more consistent travel distance of RBCs suggesting adequate precision of the paper-based LFA device for monitoring of blood coagulation.

The main clinical objective for developing a new low-cost diagnostic tool that allows blood coagulation monitoring without an expensive reader is to enable adequate management of those anticoagulated patients with INR values outside of the desired therapeutic range. A total of 28 anticoagulated patients undergoing routine INR monitoring at the St. Elizabeth Healthcare clinic were recruited for the first clinical evaluation of the fabricated LFA device. The cohort of warfarin patients who consented for this study was predominantly female, Caucasian only, between 58 and 82 years of age, with clinical INR values between 1.6 and 3.8 (see Table 1). Consistent with the main clinical focus of this paper-based LFA diagnostic, the entire patient group was stratified into four different subgroups based on INR values that were experimentally determined using the CoaguChek® XS. Figure 6 summarizes the relationship between the RBC distance measured on the fabricated LFA device and corresponding INR value determined for the each patient using the same capillary blood sample. Despite the small sample sizes of the stratified patient
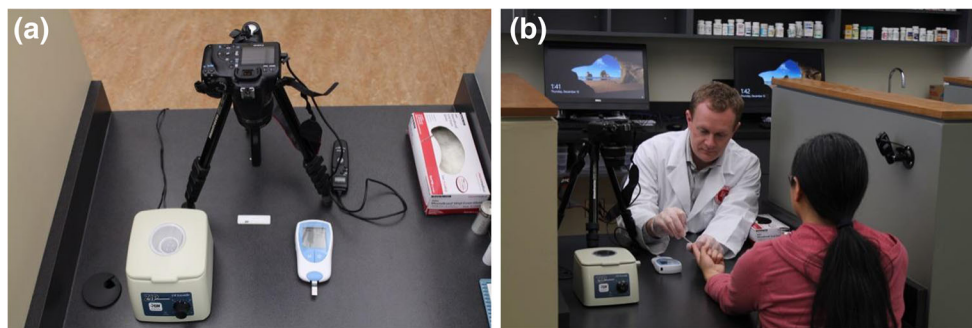

Fig. 4 Experimental setup used during clinical assessment of LFA device engineered for blood coagulation monitoring. Panel A depicts the equipment used (from left to right): Zipocrit® centrifuge to measure hematocrit of each blood sample, tripod-stabilized Canon EOS 7D digital camera vertically mounted over experimental LFA device to photographically document RBC travel distance, and CoaguChek® XS to quantify INR of each blood sample. Panel B shows Dr. M. Hegener performing a capillary blood collection on a study subject (reproduced with permission)
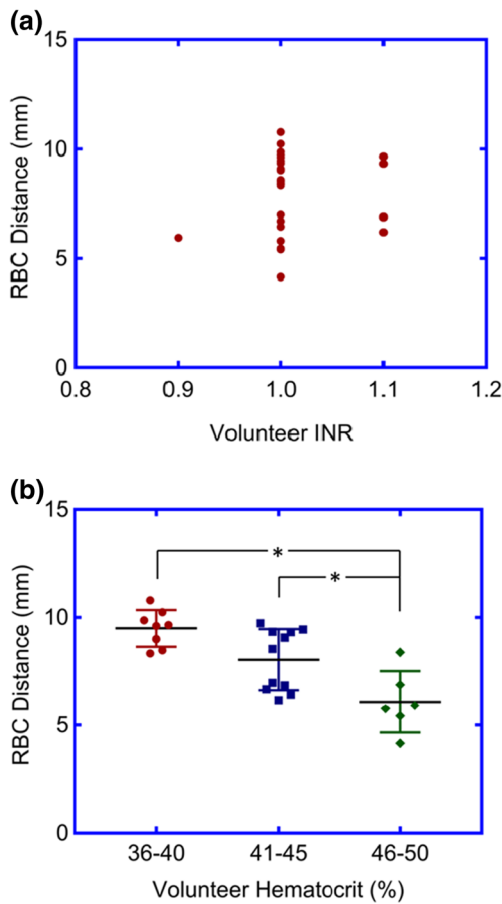
(a)

(b)

Fig. 5 Performance assessment of experimental LFA device using capillary blood samples from healthy volunteers ($n = 25$). RBC travel distance on the LFA device was measured at room temperature after 240 s using high resolution digital photographs. Panel A depicts correlation of RBC travel distance with experimentally determined INR values using the commercial CoaguChek® XS device. Panel B shows RBC travel distance as a function of subject-specific hematocrit values (see Materials and Methods) stratified into three different groups. Statistical comparison between hematocrit groups was performed by one-way ANOVA using Dunnett's multiple comparisons test (* = significantly different, $p < 0.05$)

subgroups, it is apparent that shortest RBC distance measured in each cohort positively correlates with increasing INR value. Specifically, the shortest RBC travel distance determined on the LFA device for the patient subgroup with INR values between 3.1 and 3.8 was 53.1% greater than the corresponding value measured in the patient subgroup with INR values between 1.6 and 2.0 (9.503 mm vs. 6.205 mm, respectively). These results imply that warfarin-induced anticoagulation seems to reduce overall hemodynamic viscosity and, thus, allowing RBCs to travel a greater distance on the experimental LFA device. Statistical comparison between healthy volunteers and the different patient subgroups reveals a significant difference in RBC travel distance for anticoagulated subjects with INR values ≥2.6. However, RBCs in blood samples collected from 13 warfarin patients with INR values below this threshold were found to move within the same distance as
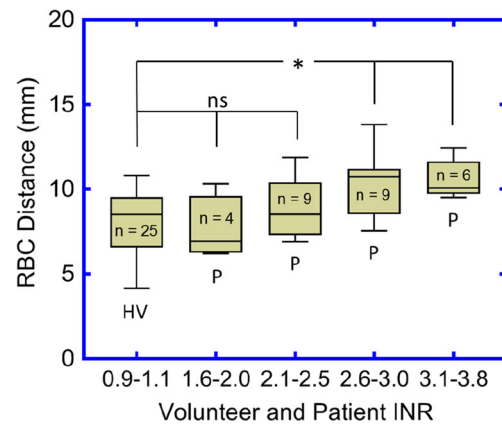

Fig. 6 Clinical validation of experimental LFA device engineered for blood coagulation monitoring. Capillary blood samples were obtained by fingerstick from healthy volunteers (HV, $n = 25$) and patients (P, $n = 28$) who were prescribed various oral warfarin dosage regimens. RBC travel distance on the LFA device was measured at room temperature after 240 s using high resolution digital photographs. INR of each blood sample was quantified using the commercial CoaguChek® XS device. Patient results are stratified according to therapeutically relevant INR values. Statistical comparison between the healthy volunteer control group and various patient groups was performed by one-way ANOVA using Dunnett's multiple comparisons test (* = significantly different from healthy volunteers, $p < 0.05$; ns = not significantly different)

measured for participants in the healthy volunteer control group. From these data, it is predicted that this low-cost, paper-based LFA device appears to have clinical utility for identifying anticoagulated patients taking vitamin K antagonists who are outside the desired therapeutic efficacy window and should follow-up with a health care provider for quantitative INR determination in order to adjust anticoagulant dose.

4 Discussion

The scientific rationale underlying utilization of a LFA device for clinical blood coagulation monitoring is based upon changes in whole blood viscosity observed during the coagulation process (Ranucci et al. 2014). Physiological hemostasis is effectively achieved through a complex interplay between the vascular system, coagulation system, fibrinolytic system and platelets. Activation of the intrinsic or extrinsic coagulation pathway changes the physical properties of blood from a viscoelastic fluid to a viscoelastic solid after formation of a cross-linked fibrin clot, with whole blood viscosity progressively increasing during this process (Errill 1969). To explore the relationship between travel time on the experimental LFA device and the dynamic viscosity during blood coagulation, the time required for the solvent front of various glycerol/water mixtures to reach the end of the 16.5 mm observation window was measured. Since the viscosity values of the glycerol/water mixtures selected for this *in vitro* test were within the reported range for coagulating blood under low

shear stress, the direct proportionality depicted in Fig. 3 between travel time and viscosity demonstrates scientific compliance of the flow properties on the fabricated LFA device with the theoretical paradigm of fluid flow within a horizontal capillary as defined by Lucas, Washburn, and Rideal in the 1920s (Washburn 1921, Rideal 1922). These results are consistent with earlier preclinical data reported by our research groups where the effect of controlled, calcium-dependent blood coagulation was measured using paper-based LFA devices (Li et al. 2014, Li et al. 2017).

The LFA device is not intended to entirely replace traditional INR monitoring, but rather serve as a qualitative tool for patient self-testing to determine whether a follow-up visit with a healthcare provider for a quantitative INR assessment is necessary. Patient self-testing of INR using POC devices is already an established monitoring modality with improvements observed in time in therapeutic range, quality of life, and satisfaction as compared to traditional INR monitoring. Unfortunately, the high cost of currently available monitoring devices is a limitation to patient self-testing (Phibbs et al. 2016).

To optimize the device for clinical use, correlation of RBC travel distance at 240 s with the therapeutically desired INR range of 2.0 to 3.0 for most indications must be established (Coumadin® 2017). In this clinical feasibility study, the desired INR range corresponded to a mean RBC front travel distance range of 8.87 ± 1.66 mm to 10.26 ± 1.78 mm. On the LFA device, this is visually represented by the qualitative "OK" range (Fig. 1). If the RBC front ends within the "OK" range after 240 s, the patient's INR would be expected to fall within the 2.0 to 3.0 desired range. Based upon the preliminary results of this study, sending patients with RBC front travel distance values corresponding to INR > 2.6 for a more quantitative assessment seems a conservative but prudent approach due to the device's ability to find a statistically significant difference in INR value at this point (Fig. 6). Based on the results from this pilot study, it is conceivable to determine a more precise "OK" RBC front travel distance range. To achieve 80% power in such a pivotal study, it was calculated that 20–30 participants will need to be recruited per stratified INR group.

Clinical use of an optimized version of this LFA device may lead to significant healthcare cost and resource savings. Patients on warfarin therapy make frequent visits for INR monitoring, with a maximum recommended interval between appointments of 4–12 weeks for stabilized patients. It is estimated that 55–64% of patients in the United States are in their desired INR range when tested (Mearns et al. 2014). Therefore, over one-half of testing appointments are arguably unnecessary, leading to preventable costs and patient inconvenience. If patients had access to an inexpensive, qualitative test, such as this LFA device, testing could be performed at home with health care provider visits made for results outside of the defined "OK" range. With an average INR monitoring cost of $69.76 per test, this could result in substantial savings (Anderson et al. 2015). Costs associated with transportation to INR monitoring sites must also be considered, with the average round-trip transportation cost for those living in urban environments being $10.78 (Hwang et al. 2011). If a patient visits a health care provider for INR checks every 4 weeks (12–13 tests annually) and one-half of these tests are unnecessary because INR is in the desired range, use of a qualitative test, such as this LFA device, could reduce annual healthcare costs by over $480 per patient. In addition, fewer appointments for arguably unnecessary tests would reduce health care provider workload, providing more time for patients who need services most. This device also has the potential for use in developing countries where access to healthcare providers is more limited and technology such as electricity for the other devices is not always readily available.

The commercial availability of a low cost, LFA device may also facilitate rapid identification of patients at risk of complications due to under- or over-anticoagulation. Just a 10% increase in the time out of therapeutic range is associated with an increased risk of mortality and thromboembolic events. In atrial fibrillation, the risk of stroke is nearly 4 times higher for patients with an INR < 1.5 than those with an INR of 2.0 to 2.9 (Brass et al. 1997, Jones et al. 2005). Achieving a greater time in the therapeutic range has been demonstrated with regular INR monitoring with timely dose adjustments (Wieloch et al. 2011). Weekly INR self-testing with currently available POC devices has demonstrated improvements in time in therapeutic range (Phibbs et al. 2016). Patients could be instructed to test with a LFA device weekly (instead of the typical 4 week gap in between routine INR checks) and report to a healthcare provider for a quantitative INR check with any result out of the "OK" range.

This small-scale, clinical feasibility study has limitations that will need to be considered when designing subsequent pivotal studies. Participant use of concomitant medications known to affect blood viscosity (e.g., nonsteroidal anti-inflammatory drugs and certain dietary supplements) was not determined and any effect these agents have on blood viscosity independent of INR may have confounded the results. In addition, the highest INR value included in the study was 3.8, which is not far from the desired therapeutic range for most patients. Stratified data analysis within the healthy volunteer group using hematocrit as a factor demonstrated significantly slower RBC movement on the experimental LFA device when blood samples contained a greater fraction of RBCs. This result strongly implies that hematocrit is a confounding variable that may need to be minimized during future device optimization. A ten-percent increase in hematocrit has been shown to increase blood viscosity by approximately 5%. Fibrinogen and immunoglobulins are also known to influence whole blood viscosity, but not to the same extent as the hematocrit (Cho and Jung 2014).

The LFA device may have utility beyond monitoring anticoagulation therapy with vitamin K antagonists, such as warfarin. Future research could also investigate RBC front travel distance on the LFA device among participants taking the newer oral anticoagulants such as dabigatran, rivaroxaban, apixiban, or edoxaban, which do not have readily available monitoring methods. Since the RBC front travel distance on the LFA device directly correlates with the overall changes of coagulation-induced blood viscosity irrespective where the pharmacological interference within the coagulation cascade occurs, this novel, low-cost diagnostic device may prove to be a valuable monitoring in a broader patient population.

5 Conclusion

LFA devices have many favorable characteristics for POC blood coagulation testing. This feasibility study demonstrated that RBC front travel distance from 30 μL of whole blood responds to changes in INR. These results suggest that anticoagulation induced by warfarin reduces overall hemodynamic viscosity and, thus, allows RBCs to travel a longer distance at a fixed time on the experimental LFA device. Statistical analysis reveals a significant difference in RBC travel distance between healthy volunteers and the patient subgroups with INR values ≥2.6. Since the hematocrit has an effect on RBC travel distance on LFA, further optimization of this device may be necessary to increase robustness of the qualitative monitoring data used for health care providers to implement therapeutically relevant dosage adjustments.

References

CAP TODAY, Coagulation analyzers—point of care, self-monitoring (College of American Pathologists, Northfield, IL; March (2017), http://www.captodayonline.com/productguides/instruments/coagulation-analyzers-march-2017.html. Accessed April 4, (2017)

CoaguChek XS PT Test [package insert]. Roche Diagnostics, Indianapolis, IN; January. (2016). http://www.coaguchek-usa.com/content/dam/internet/dia/coaguchek/coaguchek-usa_com/coaguchek_hcp/pdf/CoaguChek-XS-Package-Insert-2016.pdf. Accessed April 17, 2017

Coumadin® [package insert]. (Bristol-Myers Squibb Company, Princeton, 2017); May. 2017. https://packageinserts.bms.com/pi/pi_coumadin.pdf. Accessed June 21, 2017

E. Anderson, K. Johnke, D. Leedahl, M. Glogoza, R. Newman, C. Dyke, Am. J. Surg. **210**, 1095 (2015)

J. Ansell, J. Hirsh, E. Hylek, A. Jacobson, M. Crowther, G. Palareti, Chest **133**, 160S (2008)

H.E. Bloomfield, A. Krause, N. Greer, et al., Ann. Intern. Med. **154**, 472 (2011)

L.M. Brass, H.M. Krumholz, J.M. Scinto, M. Radford, Stroke **28**, 2382 (1997)

E. Chiquette, M.G. Amato, H.I. Bussey, Arch. Intern. Med. **158**, 1641 (1998)

Y.I. Cho, J.M. Jung, Int. Commun. Heat. Mass. Transf. **57**, 221 (2014)

J.W. Eikelboom, D.J. Quinlan, J. van Ryn, J.I. Weitz, Circulation **132**, 2412 (2015)

E.W. Errill, Physiol. Rev. **49**, 863 (1969)

K.R. Hawkins, B.H. Weigl, Proc. SPIE (2010). doi:10.1117/12.845500

P.L. Hess, M.J. Mirro, H.-C. Diener, J.W. Eikelboom, S.M. Al-Khatib, E.M. Hylek, H.B. Bosworth, B.J. Gersh, D.E. Singer, G. Flaker, J.L. Mega, E.D. Peterson, J.S. Rumsfeld, B.A. Steinberg, A.K. Kakkar, R.M. Califf, C.B. Granger, Am. Heart J. **168**, 239 (2014)

J.M. Hwang, J. Clemente, K.P. Sharma, T.N. Taylor, C.L. Garwood, J. Manag. Care. Spec. Pharm. **17**, 635 (2011)

M. Jones, P. McEwan, C.L. Morgan, J.R. Peters, J. Goodfellow, C.J. Currie, Heart **91**, 472 (2005)

C. Kearon, E.A. Akl, J. Ornelas, A. Blaivas, D. Jimenez, H. Bounameaux, M. Huisman, C.S. King, T.A. Morris, N. Sood, S.M. Stevens, J.R.E. Vintch, P. Wells, S.C. Woller, L. Moores, Chest **149**, 315 (2016)

H. Li, D. Han, M.A. Hegener, G.M. Pauletti, A.J. Steckl, Biomicrofluidics **11**, 024116 (2017)

H. Li, D. Han, G.M. Pauletti, A.J. Steckl, Lab Chip **14**, 4035 (2014)

R. Lozano, M. Naghavi, K. Foreman, S. Lim, K. Shibuya, V. Aboyans, J. Abraham, T. Adair, R. Aggarwal, S.Y. Ahn, M.A. AlMazroa, M. Alvarado, H.R. Anderson, L.M. Anderson, K.G. Andrews, C. Atkinson, L.M. Baddour, S. Barker-Collo, D.H. Bartels, M.L. Bell, E.J. Benjamin, D. Bennett, K. Bhalla, B. Bikbov, A.B. Abdulhak, G. Birbeck, F. Blyth, I. Bolliger, S. Boufous, C. Bucello, M. Burch, P. Burney, J. Carapetis, H. Chen, D. Chou, S.S. Chugh, L.E. Coffeng, S.D. Colan, S. Colquhoun, K.E. Colson, J. Condon, M.D. Connor, L.T. Cooper, M. Corriere, M. Cortinovis, K.C. de Vaccaro, W. Couser, B.C. Cowie, M.H. Criqui, M. Cross, K.C. Dabhadkar, N. Dahodwala, D. De Leo, L. Degenhardt, A. Delossantos, J. Denenberg, D.C. Des Jarlais, S.D. Dharmaratne, E.R. Dorsey, T. Driscoll, H. Duber, B. Ebel, P.J. Erwin, P. Espindola, M. Ezzati, V. Feigin, A.D. Flaxman, M.H. Forouzanfar, F.G.R. Fowkes, R. Franklin, M. Fransen, M.K. Freeman, S.E. Gabriel, E. Gakidou, F. Gaspari, R.F. Gillum, D. Gonzalez-Medina, Y.A. Halasa, D. Haring, J.E. Harrison, R. Havmoeller, R.J. Hay, B. Hoen, P.J. Hotez, D. Hoy, K.H. Jacobsen, S.L. James, R. Jasrasaria, S. Jayaraman, N. Johns, G. Karthikeyan, N. Kassebaum, A. Keren, J.-P. Khoo, L.M. Knowlton, O. Kobusingye, A. Koranteng, R. Krishnamurthi, M. Lipnick, S.E. Lipshultz, S.L. Ohno, J. Mabweijano, M.F. MacIntyre, L. Mallinger, L. March, G.B. Marks, R. Marks, A. Matsumori, R. Matzopoulos, B.M. Mayosi, J.H. McAnulty, M.M. McDermott, J. McGrath, Z.A. Memish, G.A. Mensah, T.R. Merriman, C. Michaud, M. Miller, T.R. Miller, C. Mock, A.O. Mocumbi, A.A. Mokdad, A. Moran, K. Mulholland, M.N. Nair, L. Naldi, K.M.V. Narayan, K. Nasseri, P. Norman, M. O'Donnell, S.B. Omer, K. Ortblad, R. Osborne, D. Ozgediz, B. Pahari, J.D. Pandian, A.P. Rivero, R.P. Padilla, F. Perez-Ruiz, N. Perico, D. Phillips, K. Pierce, C.A. Pope, E. Porrini, F. Pourmalek, M. Raju, D. Ranganathan, J.T. Rehm, D.B. Rein, G. Remuzzi, F.P. Rivara, T. Roberts, F.R. De León, L.C. Rosenfeld, L. Rushton, R.L. Sacco, J.A. Salomon, U. Sampson, E. Sanman, D.C. Schwebel, M. Segui-Gomez, D.S. Shepard, D. Singh, J. Singleton, K. Sliwa, E. Smith, A. Steer, J.A. Taylor, B. Thomas, I.M. Tleyjeh, J.A. Towbin, T. Truelsen, E.A. Undurraga, N. Venketasubramanian, L. Vijayakumar, T. Vos, G.R. Wagner, M. Wang, W. Wang, K. Watt, M.A. Weinstock, R. Weintraub, J.D. Wilkinson, A.D. Woolf, S. Wulf, P.-H. Yeh, P. Yip, A. Zabetian, Z.-J. Zheng, A.D. Lopez, C.J.L. Murray, Lancet **380**, 2095 (2012)

E.S. Mearns, C.G. Kohn, J.-S. Song, J. Hawthorne, J. Meng, C.M. White, M.K. Raut, J.R. Schein, C.I. Coleman, Thromb. Res. **134**, 310 (2014)

S.I. O'Shea, M.O. Arcasoy, G. Samsa, S.E. Cummings, E.H. Thames, R.S. Surwit, T.L. Ortel, J. Thromb. Thrombolysis **26**, 14 (2008)

I.M. Ogilvie, N. Newton, S.A. Welner, W. Cowell, G.Y.H. Lip, Am. J. Med. **123**, 638 (2010)

P.T. Onundarson, C.W. Francis, O.S. Indridason, D.O. Arnar, E.S. Bjornsson, M.K. Magnusson, S.J. Juliusson, H.M. Jensdottir, B. Vidarsson, P.S. Gunnarsson, S.H. Lund, B.R. Gudmundsdottir, Lancet Haematol. **2**, e231 (2015)

C.S. Phibbs, S.R. Love, A.K. Jacobson, R. Edson, P. Su, L. Uyeda, D.B. Matchar, J. Gen. Intern. Med. **31**, 1061 (2016)

D. Pugh, J. Pugh, G.E. Mead, Age Ageing **40**, 675 (2011)

M. Ranucci, T. Laddomada, M. Ranucci, E. Baryshnikova, Physiol. Rep. **2**, e12065 (2014)

E.K. Rideal, Philos. Mag. **44**, 1152 (1922)

C.A. Schneider, W.S. Rasband, K.W. Eliceiri, Nat. Methods **9**, 671 (2012)

J.B. Segur, H.E. Oberstar, Ind. Eng. Chem. **43**, 2117 (1951)

B.S. Stambler, L.M. Ngunga, Int. J. Gen. Med. **8**, 231 (2015)

E.W. Washburn, Phys. Rev. **17**, 273 (1921)

M. Wieloch, A. Själander, V. Frykman, M. Rosenqvist, N. Eriksson, P.J. Svensson, Eur. Heart J. **32**, 2282 (2011)

J.J. You, D.E. Singer, P.A. Howard, D.A. Lane, M.H. Eckman, M.C. Fang, E.M. Hylek, S. Schulman, A.S. Go, M. Hughes, F.A. Spencer, W.J. Manning, J.L. Halperin, G.Y.H. Lip, Chest **141**, e531S (2012)