High-resolution musculoskeletal ultrasound with power Doppler (MSKUS/PD) for hemophilic mouse joints provides a non-invasive model to study location-specific clustering of vascular changes associated with joint bleeding in hemophilia

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Introduction

Patients with hemophilia A (PWH) or B, caused by deficiency of Factor (F)VIII or FIX respectively, suffer from recurrent bleeds into 'target joints'. In approximately 30-50% of PWH, these target joints develop arthropathy later in life, despite the patient undergoing prophylaxis since childhood[1].

Hemophilic arthropathy (HA) is a disabling condition characterized by joint deformity with chronic synovial proliferation, increased vascularity, hemosiderin deposition, villus formation and cartilage destruction[2,3].

On the whole, the cause of repeated joint bleeds in PWH and the underlying mechanisms of HA progression remain poorly understood.

Persistent vascular remodeling and vessel "leakiness" were recently identified as important features of HA that may contribute to re-bleeding and perpetuated bleeding[4,5]. However, it is not yet known whether these vascular changes are uniformly distributed in the joint.

These observations provide incentive to elucidate further the mechanisms of HA, including processes of vascular remodeling, and to develop new treatments that specifically target angiogenesis in the joint.

Here, we use **high resolution musculoskeletal ultrasound with Power Doppler (MSKUS/PD)** to explore clustering of microvascular changes in human knee joints during hemarthrosis, and apply this method to a mouse model of hemophilic joint disease.

Objectives

1. To study vascular changes associated with knee bleeding in PWH and establish whether or not these are uniformly distributed.

2. To adapt and validate MSKUS/PD for detection of vascular perfusion in murine knee joints, and thereby to demonstrate that hemarthrosis-associated vascular changes in hemophilic mice emulate human pathology.

Methods

1. Patient studies: Four PWH were imaged by high resolution MSKUS/PD at baseline and during acute bleeding episodes in the knee joint. MSKUS/PD imaging was performed in four locations: medial meniscus (MM), medial recess (MR), lateral recess (LR), and suprapatellar fat pad (SPFP). A GE Logiq S8 model was used with transducer frequencies of 8-16 MHz and standardized scanning protocols were applied[6].

2. Murine studies: MSKUS/PD was performed using a GE Logiq e model with settings adapted for murine anatomy. Optimal results were achieved with the Small Parts preset and a transducer frequency of 10 MHz. Sensitivity of mouse-specific MSKUS/PD settings to detect microcirculatory perfusion changes was validated in the murine knee joint and human index finger at room temperature, after heating with pre-warmed (45°C) ultrasound gel for 30 seconds, and after cooling with ice for 30 seconds. These same MSKUS/PD settings were applied for imaging of human index fingers.

Hemarthrosis was induced in hemophilic (FVIII-deficient) mice by sub-patellar knee puncture. Vascular perfusion was assessed in the medial meniscal area by MSKUS/PD at baseline and 2 weeks after injury, and the signal quantified using ImageJ. Histology was performed at 2 weeks after injury in order to count and measure vessels by Safranin-O-fast-green staining. MSKUS/PD and histology results were correlated using Spearman's correlation analysis.

Results

- In PWH, acute knee bleeding was associated with a 4.5-fold increase in total vascular perfusion compared to baseline (n=4) (figure 1). On a location-specific basis, this increase was most prominent in the medial meniscus, followed by the medial and lateral recesses. No change was observed in suprapatellar soft tissues.

- MSKUS/PD adapted for imaging of mouse knee joints proved sensitive to fluctuations in vascular perfusion. This was evidenced by a notable rise in signal upon heating, and a decline in signal upon cooling, in both the mouse knee joint and human index finger (figure 2).

- Vascularity in knee joints of FVIII-deficient mice after induced hemarthrosis was analyzed by mouse-adapted MSKUS/PD and histology. Similar to PWH, FVIII-deficient mice exhibited a 4.2-fold increase in MSKUS/PD signal in the medial meniscus, and a 2.7-fold increase in vessel number, as determined by histology (n=5) (figure 3).

- Signal quantified from MSKUS/PD images positively correlated with two histology parameters: i) vessel number x vessel diameter (r=0.46; p=0.03) and ii) percentage of vessels ≥15 μm in diameter (r=0.47; p=0.04) (n=21) (figure 4). This validates the use of MSKUS/PD for detection of vascular changes in mice.
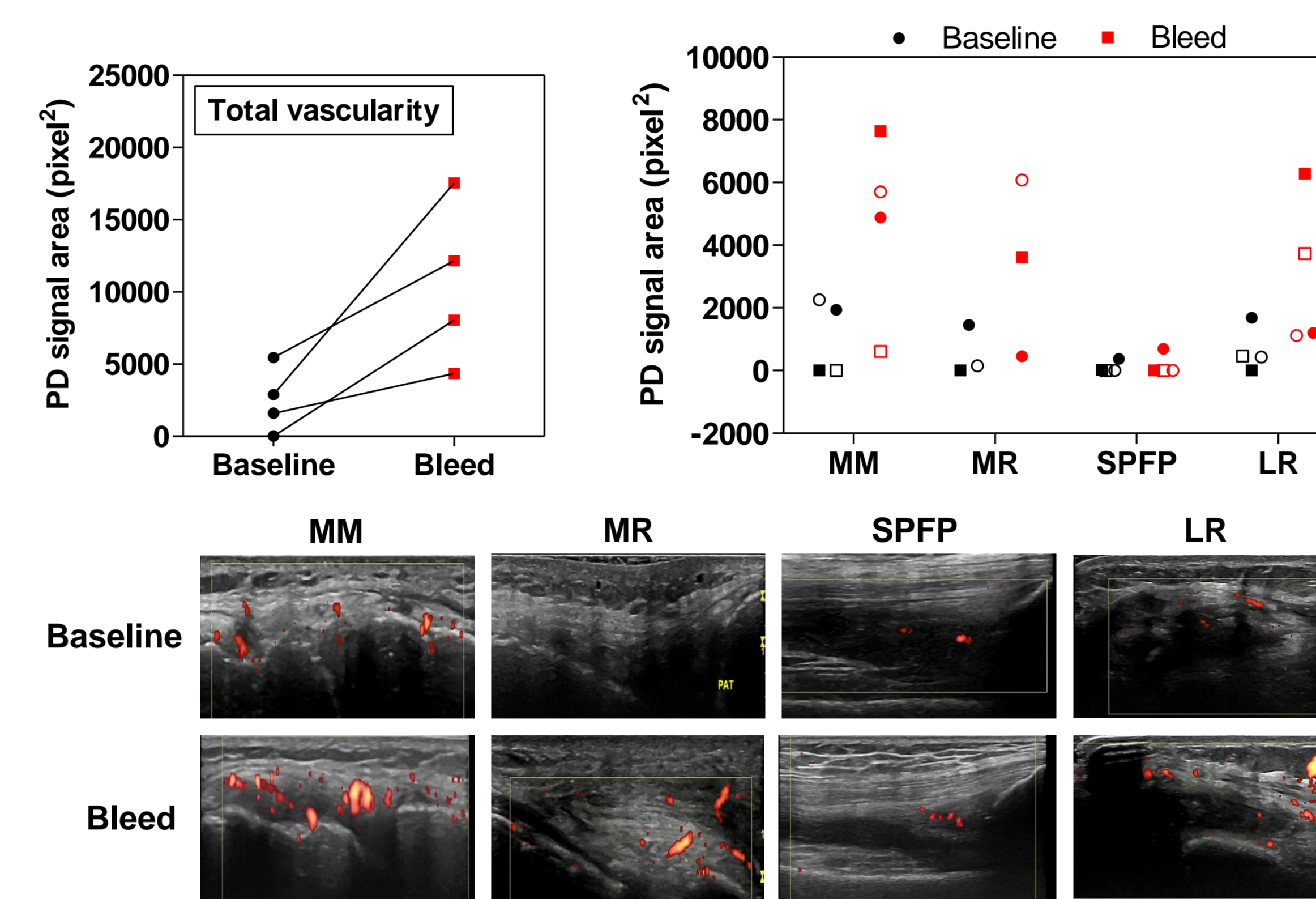

Figure 1. Location-specific increases in vascular perfusion in the knee joints of PWH during acute bleeding episodes. Upper right panel: symbols (● ○ ■ □) represent individual patients.
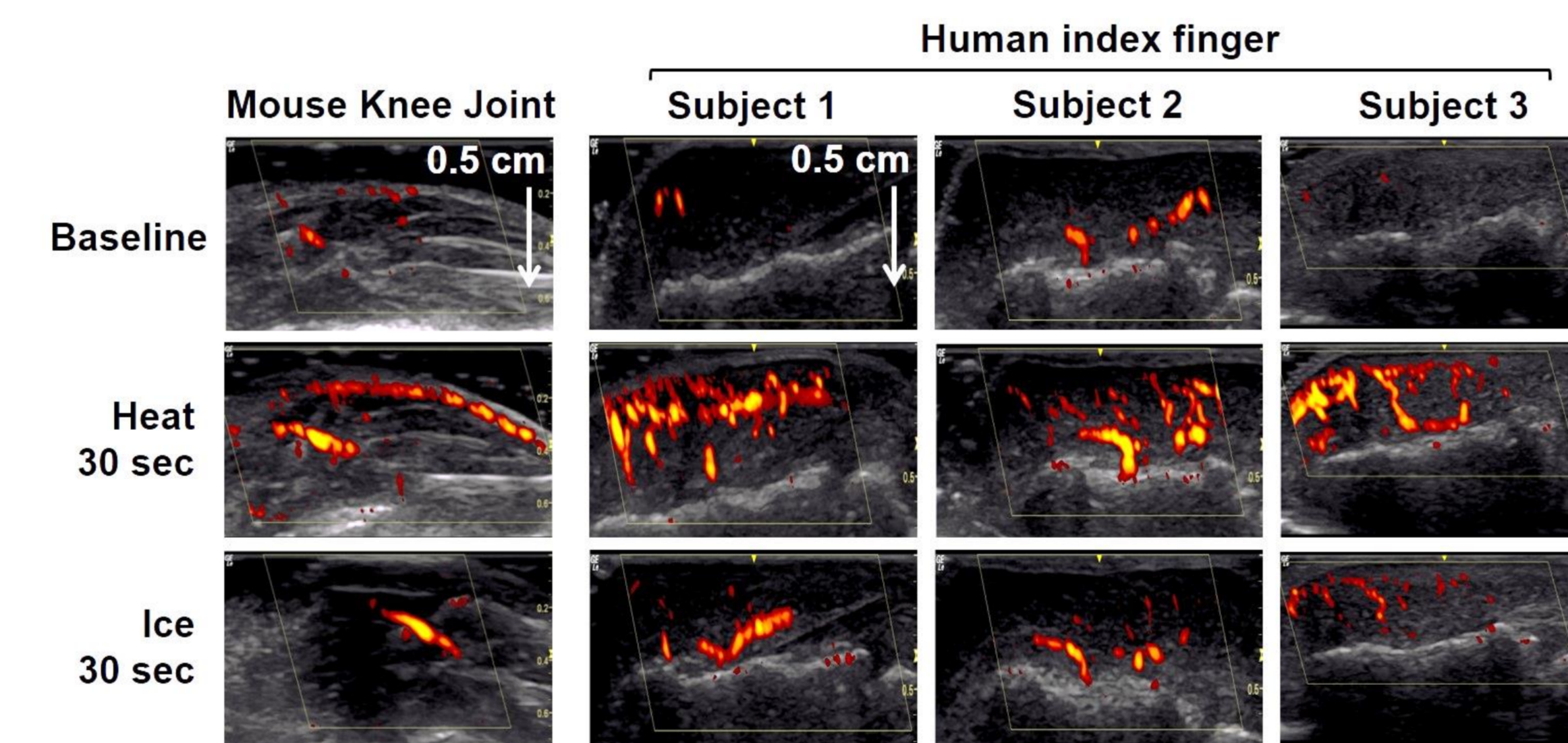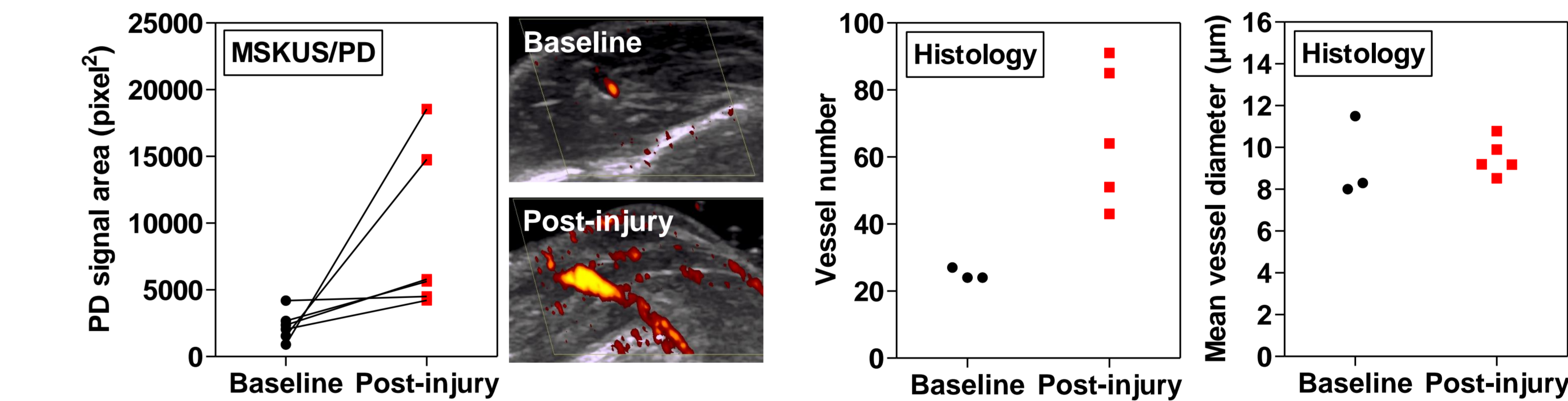

Figure 2. Sensitivity of mouse-specific MSKUS/PD settings to vascular perfusion.

Figure 3. Increased vascularity in the knee joints of FVIII-deficient mice after induced bleeding.
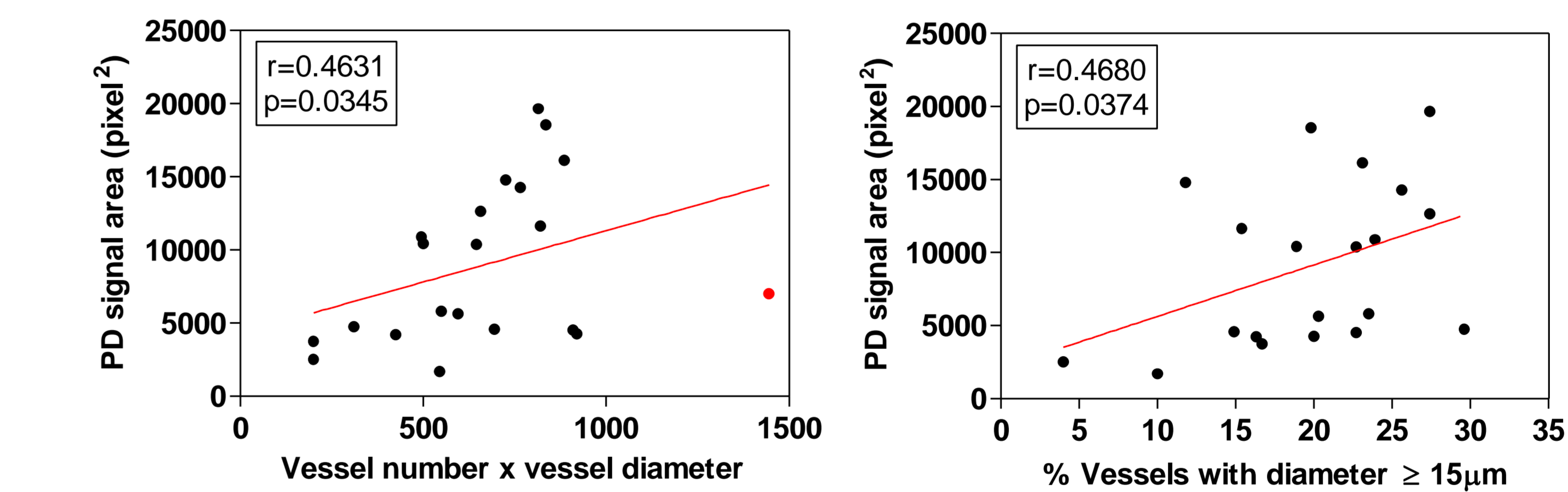

Figure 4. Correlation of vascularity quantified by MSKUS/PD and histology in FVIII-deficient mice with and without induced knee bleeding. Left panel: ● represents significant outlier (p<0.05).

Summary

1. Bleeding episodes in knee joints of PWH and FVIII-deficient mice were associated with increased vascular perfusion.

2. Vascular changes in knee joints of PWH were most prominent in the medial meniscal area, demonstrating predilection for anatomical locations. This may be important in the mechanics of re-bleeding.

3. In murine knee joints, adapted MSKUS/PD was highly sensitive to rapid vascular changes and correlated with histology data.

4. Vascular perfusion was significantly increased in the medial menisceal area of FVIII-deficient mice after induced knee bleeding, emulating human pathology.

Conclusion

MSKUS/PD can be applied for non-invasive, longitudinal studies of vascular changes in a model of joint bleeding in FVIII-deficient mice. This model will enable us to study further the pattern of vascular remodeling associated with hemarthosis in hemophilia, and aid development of novel anti-angiogenic agents to combat the development of HA.

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