Ultrasonography for in vivo monitoring of ferric chloride-induced deep vein thrombosis in rats

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INTRODUCTION

Ultrasound technology is a cost-effective, reliable, safe and accurate technique that can be used for in vivo imaging of thrombosis small rodents (mice and rats), but it often requires sophisticated high-frequency ultrasound systems or application of contrast agents. The aim of this study was to explore the possibility of using a simple generally available ultrasound system for continuous step by step visualization of thrombus formation in a ferric chloride induced deep vein thrombosis model on rats [1-3].

MATERIALS AND METHODS

All manipulations with animals were approved by SPCPA Bioethical Committee. Outbreed white male rats (n=10), body weight 300-350 g, were used in all the experiments. Rats were anesthetized by intraperitoneal injection of chloral hydrate solution in 0.9% saline (300 mg/kg of body weight). The abdomen of the rat was clipped and wiped with 70% denatured alcohol. A midline laparatomy incision was made. The region of inferior vena cava (IVC) infra renal vessels was separated form the surrounding tissues. Thrombosis was induced by application of a 2x4 mm filter paper (fig. 1) soaked in 50% ferric chloride ($FeCl_3$) solution directly on infrarenal IVC for 10 minutes.

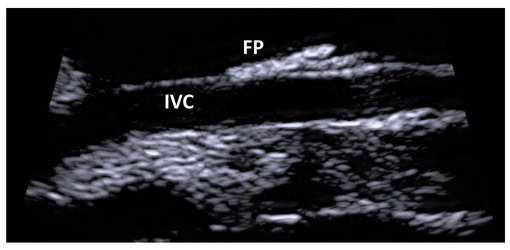

Figure 1. Filter paper soaked in 50% ferric chloride solution placed on the IVC

Filter paper was removed after the incubation period. Filter paper saturated with 0.9% saline was used for control group animals. Ultrasound contact gel was applied onto the exposed IVC. The IVC was imaged using MyLabTouch ultrasound system (Esaote, Italy) with a 20 MHz transducer [4]. Images and video clips were obtained in gray scale B-mode, color Doppler (CD) and power Doppler (PWD) mode every 10 minutes, total observation time was 90 minutes.

RESULTS

Maximal thrombus area (in axial and transversal views), width, length of the clot and the intensity and velocity of blood flow in the IVC were monitored over time. Animals in the control group (n=5) did not develop any detectable thrombi during the observation period, there were no significant changes in blood flow parameters as well. With ferric chloride treated group (n=5) we found that active thrombus propagation starts approximately 10 minutes after injury. The size of the clot rapidly increases to reach a plateau in 60 minutes. Figure 2 presents ultrasound images obtained for consecutive stages of clot formation. Figure 3 summarizes dynamical changes in the values of the clot area (in axial and transversal views), clot length and width over time.

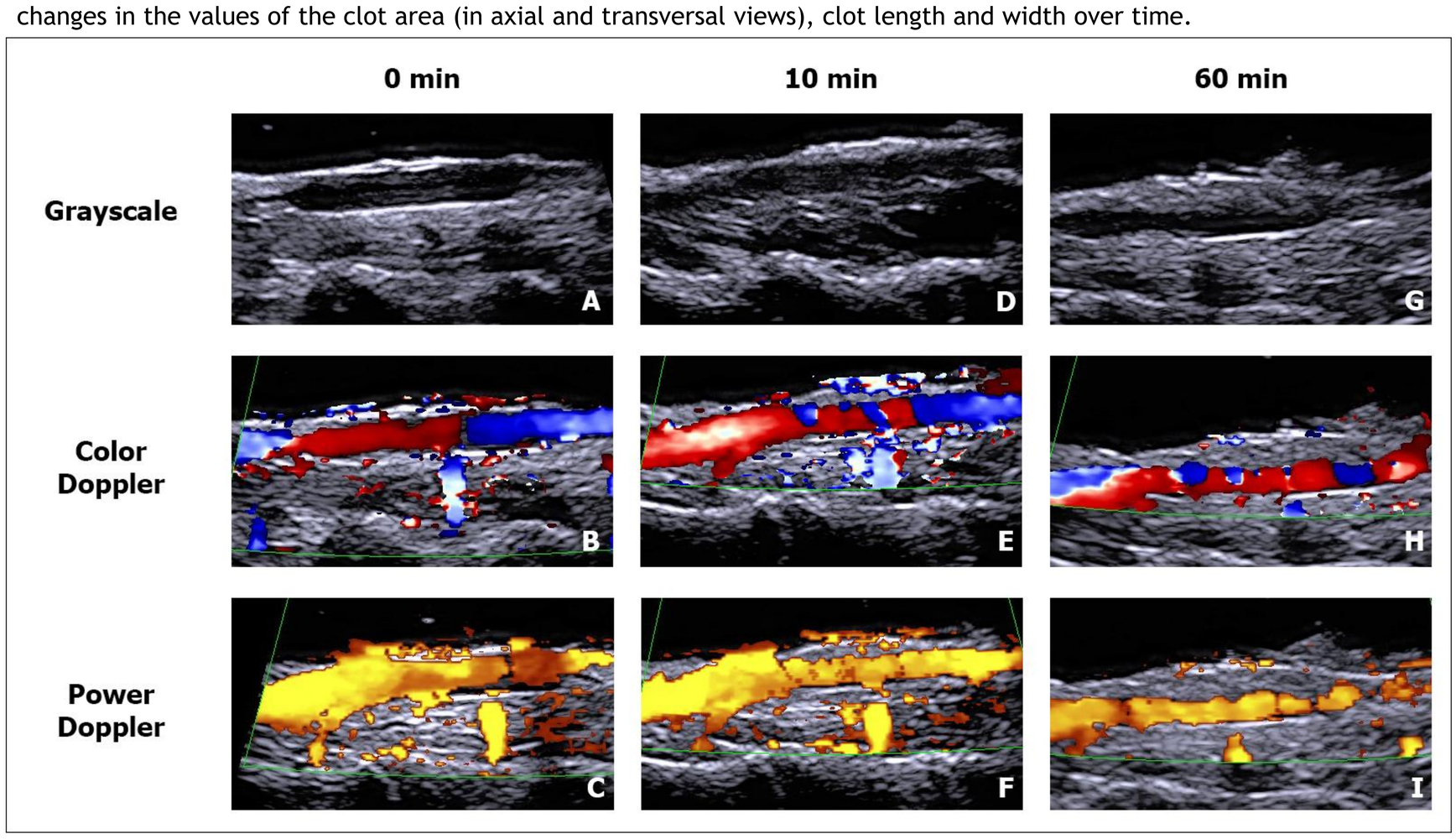

Figure 2. Monitoring of the thrombus formation in the IVC by the ultrasound system

Figure 2(A-C) includes normal images of the rat IVC immediately after filter paper removal. (D-F) Images of the IVC 10 minutes after ferric chloride application was removed. The growing clot is clearly visible under the vessel wall. The area occupied by clot is not colored on Doppler images. (G-I) Significant occlusion of the IVC by the formed clot.

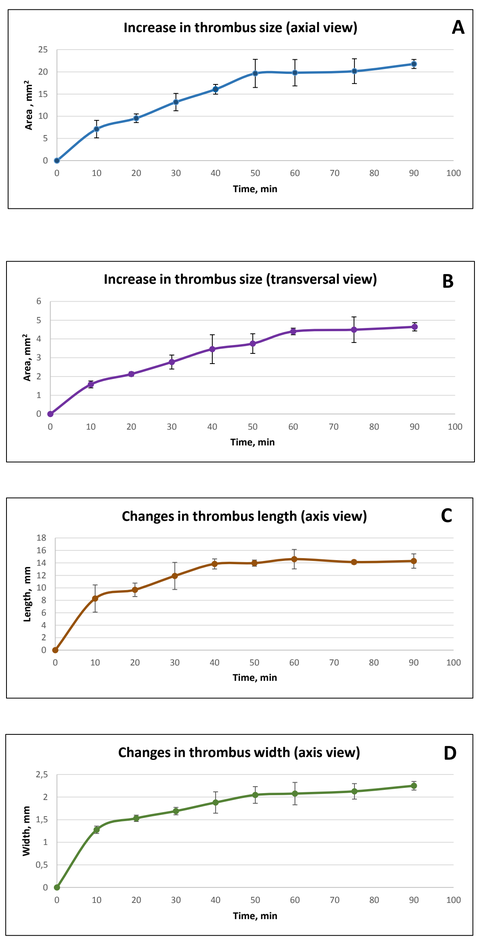

Figure 3. Changes in spatial parameters during the process of thrombus formation

CONCLUSION

Routine ultrasound imaging is suitable for real-time monitoring of thrombus formation in a rat model of ferric chloride induced thrombosis and can be applied for researching early DVT development or assessing novel antithrombotic agents.

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