Research Square

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The Effect of Nanosecond Pulsed Electric Field on Hepatic Veins—A Mid-Term Investigation

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Research

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Abstract

Background: Nanosecond pulsed electric field (nsPEF) is a novel ablative technique for treating tumours of the liver, kidneys, and other organs. This study investigated the effect of nsPEF on hepatic veins of different diameters and evaluated the feasibility and safety of using nsPEF to ablate tissues in porcine livers.

Methods: A total of 15 pigs were included in the study. B-mode ultrasonography was used to guide two-needle nsPEF electrodes inserted adjacent to the left, middle, and right hepatic veins. The animals were followed up at 1 hour and at 1, 3, 7, and 14 days post treatment.

Results: During and after the procedure of nsPEF treatment, electrocardiographs (ECGs) detected no cardiovascular events, and B-mode ultrasonography found no hepatic venous thrombosis formation. Follow-up at 1 hour to 7 days post treatment revealed complete ablation of the perivascular tissue within the ablated area. There was no apparent structural damage to the hepatic veins of different diameters, but there appeared to be red blood cells and residual inflammatory cell infiltration in the subendothelial layer from 1 hour to 7 days post treatment. Follow-up blood biochemical tests showed transient damage to the liver and myocardial function, and immediate recovery, which is consistent with the pathological changes.

Conclusion: NsPEF achieved complete liver tissue ablation while causing little damage to the hepatic veins. Thus, nsPEF could be used to ablate central tumour lesions that are located nearing the major hepatic veins, thereby avoiding some of the limitations of conventional surgical therapies and thermal ablations.

Introduction

Locoregional ablative therapies refer to nonsurgical and minimally invasive treatments that aim to destroy tumours directly. The goal of these noninvasive therapies is to reduce the pain, scarring, and mortality during treatment, while maintaining cost-effective and safe. In recent two decades, image-guided ablative therapies have been increasingly used for the local treatment of tumours. Radiofrequency ablation (RFA) is an established locoregional therapy that has been used to treat arrhythmias and chronic pain; in recent years, it has been applied clinically to ablate tumours in multiple organs, such as colorectal cancer (CRC) lung metastases [1, 2]. RFA has also been used to treat colorectal liver metastasis [3], gastric cancer liver metastases [4], renal cancer [5–7], and hepatocellular carcinoma (HCC) [8–9]. However, this technique is less effective and carries a higher risk when applied to tumours that exceed 5 cm in diameter; therefore, its use is limited in the treatment of large malignancies. More importantly, RFA is the radiofrequency needle punctured into the tumor under image guidance, and then radiofrequency waveguide is inserted into the body to generate localized heat, causing the temperature around the radiofrequency needle to rise. The final temperature of the radiofrequency needle can reach 110℃, while the denaturation will occur when the temperature of tumor cells exceeds 42 ℃. Thus, RFA is

often reported in conjunction with thermal damage to the surrounding tissue and blood vessels caused by electrical Joule heating [10]. This potential for damage creates a challenge for the treatment of tumours located close to major blood vessels and bile ducts.

Nanosecond pulsed electric field (nsPEF) is a novel ablative technique for treating tumours of the liver, kidneys, and other organs. With appropriate parameters applied, nsPEF is considered non-thermal, or in another word, will not cause heat-based cytotoxicity. Therefore, nsPEF can eliminate tumour cells avoiding thermal injury to the adjacent vessels or organs. In contrast to conventional heat-based ablative techniques, nsPEF does not cause direct Joule heating in the target region. Instead, it induces apoptosis and inhibits tumour angiogenesis. By delivering a high-voltage electric field with ultrashort pulses, nsPEF disrupts both the plasma membrane and intracellular structures such as endoplasmic membranes and mitochondria, thereby causing pure cell death. Since the duration of the nanosecond pulse is shorter than the charging time of the cell membrane [11], the pulses have almost no effect on the cell membrane and do not cause cell membrane electroporation. Unlike long pulses that cause electroporation of cell membranes, nsPEF produces extremely high-power pulses (several gigawatts) of very short duration (nanoseconds) and very high field strength (kV/cm), thus penetrating the cells and acting on the subcellular structures before the cell membrane is fully charged. Previous studies have confirmed that nsPEF does not affect the integrity of the extracellular membrane, but it has a marked impact on the internal cell structures, particularly the nucleus. In addition to the effects of a direct electric shock, nsPEF also has secondary effects on cells, causing them to release intracellular calcium and undergo cellular apoptosis [12, 13]. These features are particularly meaningful for treating tumours located near major hepatic vasculatures. To date, nsPEF has been widely used to treat a variety of tumours, including malignant melanoma [14], skin basal cell carcinoma [15], as well as skin squamous cell carcinoma [16].

HCC is the sixth most common malignancies worldwide and has a high incidence and mortality rate [17]. Currently, the prognosis of early- and mid-stage HCC mainly depends on the effectiveness of surgery. However, tumours that abut the main portal veins or hepatic veins are difficult to remove surgically. Previous studies have investigated the treatment of HCC with nsPEF since this technique does not cause direct Joel heating but induces complete cancer cell apoptosis [18–20]. The current study was conducted to elucidate the effect of this technique on blood vessels in the tumour ablation range and the resistance of blood vessels of different diameters to this ablation. We undertook a pilot study to determine the effects of nsPEF on hepatic veins of different diameters and evaluated the feasibility and safety of applying nsPEF in porcine livers.

Materials And Methods

Animal care

Fifteen pigs (50–60 kg; mean, 55 kg) were maintained by the Zhejiang Chinese Medical University Laboratory Animal Research Center. All animals received appropriate humane care from an experienced

laboratory stuff. The animal treatment protocols used in this study have been approved by the Animal Care and Use Committee of Zhejiang Chinese Medical University.

Anesthesia and experiment setting

All the pigs were treated with general anaesthesia (1.5–2% isoflurane) and a neuromuscular blockade to ensure complete muscle paralysis. The two-needle electrodes were placed with B-mode ultrasound guidance around the major hepatic veins.

Nanosecond pulsed electric field treatment

All animal ablative treatment was carried out with a pulse generator device provided by Ready Biological Technology Ltd. (Hangzhou, Zhejiang, China). The nsPEF treatment parameters were 500 pulses of 25 kV delivered at the absolute myocardial refractory period of the pigs (after the R-wave on the electrocardiograph [ECG]) to prevent heart arrhythmias, and the pulse frequency was 0.5 Hz. Multiple nsPEF lesions with a 1–1.5 cm spacing between the two electrodes were created in the pig livers. Three nsPEF lesions per pig were produced, and targeted to the central part of the liver directly adjacent to the hepatic veins.

Blood tests follow-up

Blood samples were collected 1 hour prior to treatment to identify baseline parameters. After treatment, blood samples were collected periodically (1 hour, 1 day, 3 days, 7 days, and 14 days post treatment) to monitor liver function, myocardial function, coagulation function, and routine blood test parameters.

Histologic and morphometric analysis

After nsPEF treatment, the ablated liver samples were dissected and fixed with formalin prior to haematoxylin and eosin (H&E) staining in the Imaging Facility of the Core Facilities, Zhejiang University School of Medicine.

Results

Animal safety

All 15 pigs survived the procedure. There were no cases of infection, bleeding of the nsPEF-treated arteries, pneumothorax, thrombosis, or animal mortality during the entire follow-up process. The vital signs of the animals remained stable throughout the treatment period, and the animals were monitored by an on-site anaesthetist and with an ECG (Fig. 1a, b, c). Electrodes were placed near the left hepatic vein, middle hepatic vein, and right hepatic vein guided by B-mode ultrasonography. Three pigs were sacrificed at each follow-up time point (1 hour, 1 day, 3 days, 7 days, and 14 days post treatment) (Fig. 1d).

Complications

Electrodes were placed near hepatic veins of different diameters (Fig. 2). After treatment, complete hepatocyte death was found within the ablation area. Figure 2 shows distinct features in the centre of the ablation area 1 hour post treatment. We measured the diameters of the hepatic veins (Fig. 2a, e, l) within the ablation area and divided them into three groups according to diameter. One group contained veins less than 4 mm in diameter (DIA < 4 mm), one group contained veins between 4 and 6 mm in diameter (DIA 4–6 mm), and one group contained veins greater than 6 mm in diameter (DIA > 6 mm). The distance between the electrode tip and the target hepatic vein wall was strictly controlled between 1.0 to 1.5 cm (Fig. 2b, f, j) according to the ablation range (Fig. 2c, g, k). The velocity of the target hepatic veins was also measured after the treatment (Fig. 2d, h, l). There was no obvious evidence of vascular stricture or thrombus formation.

Histopathological follow-up

Figure 3 shows the gross anatomy of the dissected liver samples with distinguishing feature of the sharp ablation areas. The dissected tissues were selected based on the presence of hepatic veins of different diameters in the ablation area. Figure 4 demonstrated the pathologic images of the ablated liver tissues, which is marked by extensive cell death and haemorrhagic changes. Haemorrhage occurred at 1 hour post treatment and became more severe 1 day post treatment. After seven days, the areas of haemorrhage partially resolved and after 14 days completely resolved in the ablated area. The hepatic veins in the ablated area were affected, but not to the same extent as the liver tissues in the ablation area. The hepatic veins in all three diameter groups (DIA < 4mm: vascular diameter < 4 mm; DIA 4–6 mm: vascular diameter 4–6 mm; DIA > 6 mm: vascular diameter > 6 mm) showed the presence of red blood cells and residual inflammation in the subendothelial layer, without apparent structural destruction, from 1 hour to 7 days post treatment. Moreover, relative to the smaller hepatic veins (DIA < 4 mm and DIA 4–6 mm), the major hepatic veins (DIA > 6 mm) were less affected after the nsPEF treatment. Haemorrhagic congestion with red blood cells and the endothelial injury were less significant in the major hepatic veins than in the smaller hepatic veins at 1 and 3 days post nsPEF. However, after 14 days, the areas of traversing hepatic vein congestion in the ablated area had resolved, and the damage incurred by the various sizes of hepatic veins showed recovery.

Vital organ function follow-up

Figure 5 presents the results of the blood count follow-up, which included the following tests: white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haematocrit (HCT), and basophils count (BASO). Figure 6 presents the results of the liver function and myocardial function follow-up, which included testing for alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH). Figure 7 shows the follow-up measurement of coagulation function, which included tests for activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), prothrombin ratio (PTR), prothrombin time activity percentage (PTA), fibrinogen, and international normalized ratio (INR).

These follow-up biochemical results showed a mild inflammatory response at 1 day post treatment, and that transient liver function damage and myocardial damage had occurred, which was followed by a subsequent recovery. During the follow-up period, although the enzyme counts initially increased from baseline, enzyme levels and white blood cell counts began to decline after 3 days. After 14 days, the abnormal results had returned to baseline.

Discussion

Nanosecond pulsed electric field is a recently developed interventional radiology modality that can be used to treat a wide range of solid tumours. It does not cause direct Joule heating in the targeted region, thus spares vascular structures including veins, arteries and bile ducts, and avoids temperature-related problems such as vascular rupture and adhesion.

nsPEF has been shown to be an effective local ablative therapy for HCC treatment. Although the ablation effect of nsPEF on tumour cell death has been confirmed to occur through nano-pulse stimulation, the underlying mechanism is still unclear. The induction of tumour cell apoptosis [21–24] and anti-angiogenic effects [25] has been reported in previous studies. In 2011, Beebe et al. investigated the effect of nanosecond pulses on hepatocellular carcinoma [20]. The authors found that nsPEF could effectively treat ectopic hepatocellular carcinoma, and that the therapeutic effect was associated with changes in caspase-3 activity in tumour cells, which provides a theoretical basis for the preclinical application of orthotopic hepatocellular carcinoma. Chen et al. [14] studied the long-term survival of mice after nanosecond pulse ablation of hepatocellular carcinoma, and the mechanism by which this treatment modality exerts its effects. nsPEF ablation was shown to eliminate hepatocellular carcinoma by inducing apoptosis and inhibiting angiogenesis. These findings provide a further theoretical basis for the clinical application of nanosecond pulse ablation in hepatocellular carcinoma.

In addition, previous studies have shown that nsPEF has extensive biological effects in colon cancer [26–27], ovarian cancer [28–29], oral cancer [30–32], pancreatic cancer [33], and fibrosarcoma [34]. In addition to inducing tumour cell apoptosis, nsPEF modulates tumour cell proliferation, invasion and metastasis, the tumour microenvironment, angiogenesis, and other tumour characteristics[35–40].

The current study systemically followed up the ablation outcomes, and identified several advantages of applying nsPEF ablation: (1) The red blood cell count remained consistent, proving that massive haemorrhage did not occur during treatment. There was no significant difference between the pretreatment and post-treatment white blood cell counts, suggesting that strict asepsis during the ultrasound-guided puncture operations and nsPEF ablation could effectively prevent infection, which ensures further human-trial safety. (2) Liver enzyme levels can be used to assess the damage to liver function caused by ablation. Aminotransferase (ALT and AST) levels were temporarily elevated, which was assumed to result from hepatocytes death within the ablation area. Their subsequent recovery confirmed that the remaining liver volume is enough to compensate the damaged liver tissues, proving the safety of nsPEF. (3) In this study, we employed a synchronous cardiac pulse generation system that

automatically stopped when the ECG detected irregular cardiac activity, effectively protecting the cardiac muscles from electrical injury. The slight changes in cardiac enzymes CK and CK-MB were not induced by myocardial injury but by muscle puncture. The cardiac troponin I levels and the return of CK and CK-MB to baseline after 14 days confirmed this hypothesis. In HCC patients, muscle injury-induced CK-MB release and the mild elevation of LDH are tolerant under Child-A or -B score situation. (4) Thermal ablation reportedly increases the risk of thrombogenicity, which can lead to the administration of heparin [41]. Our results showed that the blood platelet levels were reduced post treatment, and returned to baseline after 14 days. Coagulation indexes also underwent a temporary change, and their subsequent recovery indicated that nsPEF does not result in a procoagulant effect. In addition, the histopathologic results did not indicate thrombosis. Therefore, no anticoagulant therapy is required after nsPEF treatment. These findings provide a foundation for the future clinical application of nsPEF to treat tumours.

The current study sought to elucidate the effect of this technique on blood vessels in the ablation range of the tumour and the resistance of blood vessels of different diameters to this ablation. We investigated the effect of nsPEF on hepatic veins of different diameters and evaluated the feasibility and safety of nsPEF on porcine livers. The pathological follow-up showed that the hepatic veins in the ablated area were maintained along with the complete vascular structure, while the targeted perivascular tissues were accurately ablated with no sparing. Furthermore, we found that compared with the major hepatic veins (DIA > 6 mm), the smaller hepatic veins (DIA < 4 mm and DIA 4–6 mm) were more strongly affected by nsPEF treatment. However, after 14 days, the areas of traversing hepatic vein congestion had resolved in the ablated area, and the smaller hepatic veins had recovered. The flow velocity of the target hepatic veins before and after treatment was the same, and there was no obvious evidence of aneurysm or thrombus formation. Taken together, the above results indicate that nsPEF does not have a deleterious effect on hepatic veins.

There were several limitations to this study. Although the distance between the probe and vessels and their orientation to each other were taken into account, the vessels were not targeted directly in all cases. In addition, although our study mainly focused on the influence of nsPEF on hepatic veins with different diameters, there may additionally be portal veins, bile duct, and/or arteries close to the HCC tumour mass. The safety of this technique in these veszoneareasel types is also an important consideration that requires further investigation.

Conclusions

nsPEF ablation targets liver tissue with little effect on the structure and function of the hepatic veins. This enables the application of nsPEF to ablate central tumour lesions that are located near hepatic veins, avoiding some limitations of conventional thermal ablation.

Abbreviations

nsPEF: Nanosecond pulsed electric field; RFA: Radiofrequency ablation; CRC: colorectal cancer; HCC: hepatocellular carcinoma; ECG: electrocardiogram; WBC: white blood cells; RBC: red blood cell count; PLT: platelet; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; MCH: mean corpuscular haemoglobin; HCT: haematocrit; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; CK-MB: creatine kinase-MB; LDH: lactate dehydrogenase; APTT: activated partial thromboplastin time; TT: thrombin time; PT: prothrombin time; PT R: prothrombin ratio; PT%: prothrombin time activity percentage; FBG: fibrinogen; INR: international normalized ratio.

Declarations

Acknowledgements

Xinhua Chen: improved the design of the study and provided the nsPEF equipment; Danjing Guo: completed of H&E experiment.

Authors' contributions

Yeping Dong and Jiahua Lu contributed to performed the experiments, analysisand interpretation of data and drafting of the manuscript. Xinhua Chen improved the design of the study and provided the nsPEF equipment. Zhigang Ren, Liangjie Hong and Haiyu Wang collected the samples and analysed the related data. Zhiliang Huang and Dezhi Yang contributed to performed the experiments. Haiyang Xie and Wu Zhang conceived and supervised the project.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional file.

Ethics approval and consent to participate

This study was approved by the Ethical Committee of Zhejiang Chinese Medical University Laboratory Animal Research Center (approval number IACUC-20191216-04).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

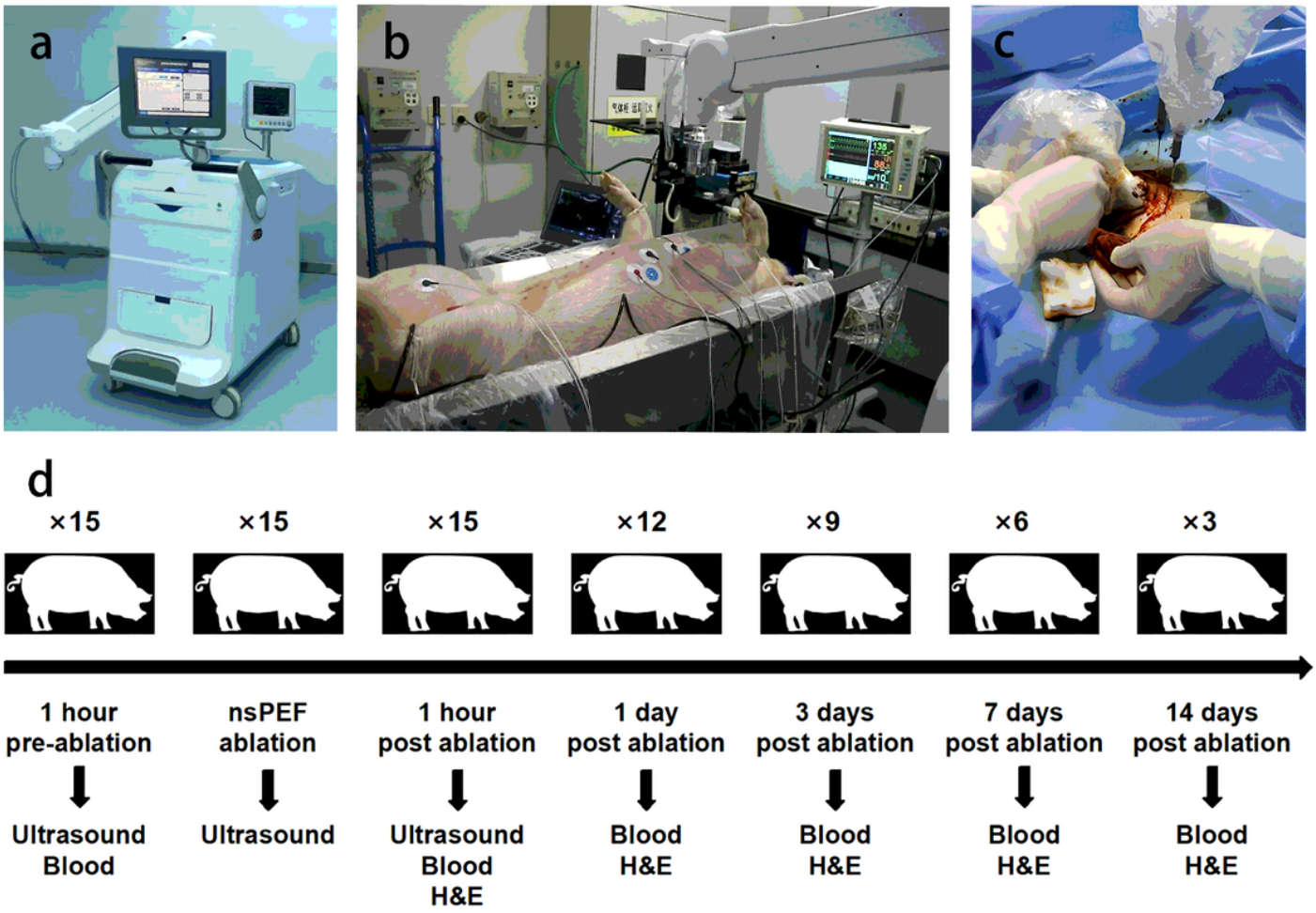


Fig. 1

Figure 1

The nanosecond pulsed electric field treatment setting. (a) Prototype of the the pulse generator device in this study. (b) Treatment was performed on an animal operating table with a pulse generator and an ECG monitor. (c) The two-needle electrodes were placed with B-mode ultrasonography guidance, and the whole procedure was maintained with general anaesthesia. (d) Flow chart of the animal experiment.

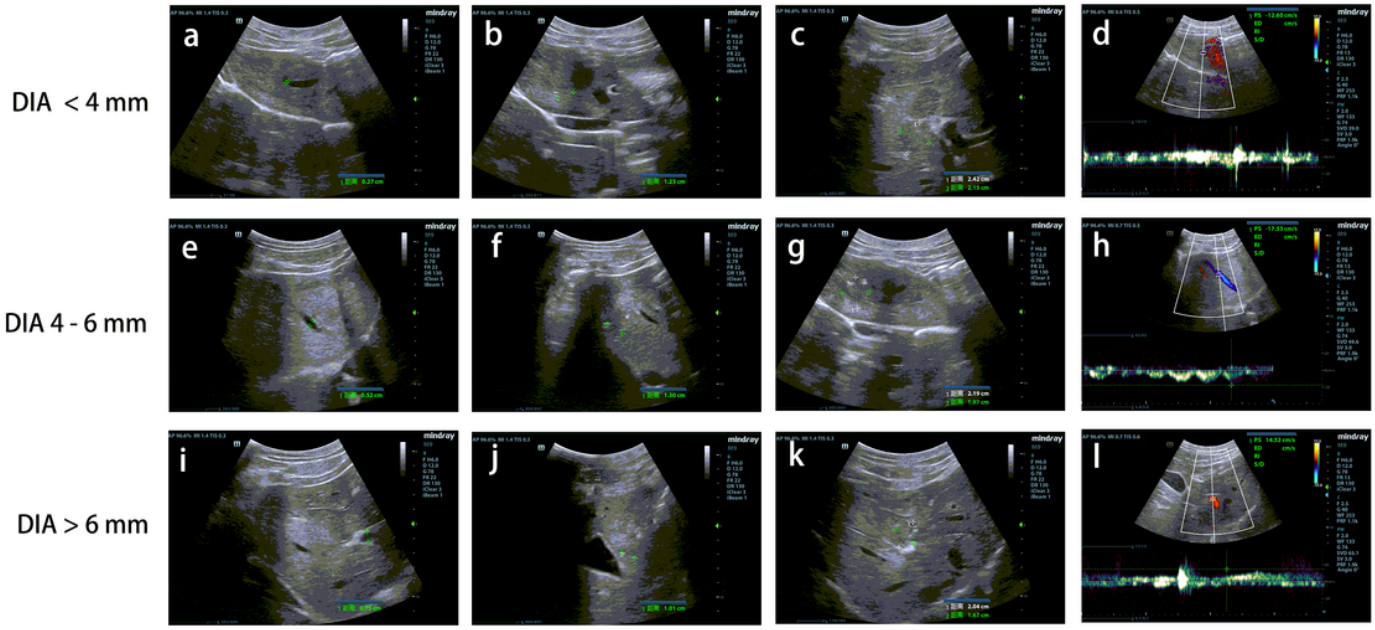


Fig. 2

Figure 2

B-mode ultrasonography images during and after treatment. (a, e, i) The diameters of the hepatic veins within the ablation area were divided into three groups according to their diameters—less than 4 mm in diameter (DIA < 4 mm); between 4 and 6 mm in diameter (DIA 4–6 mm) and greater than 6 mm in diameter (DIA > 6 mm). (b, f, j) The distance between the electrode tip and the target hepatic vein wall. (c, g, k) The size of the ablation range. (d, h, l) The blood flow velocity of the target hepatic veins after the treatment.

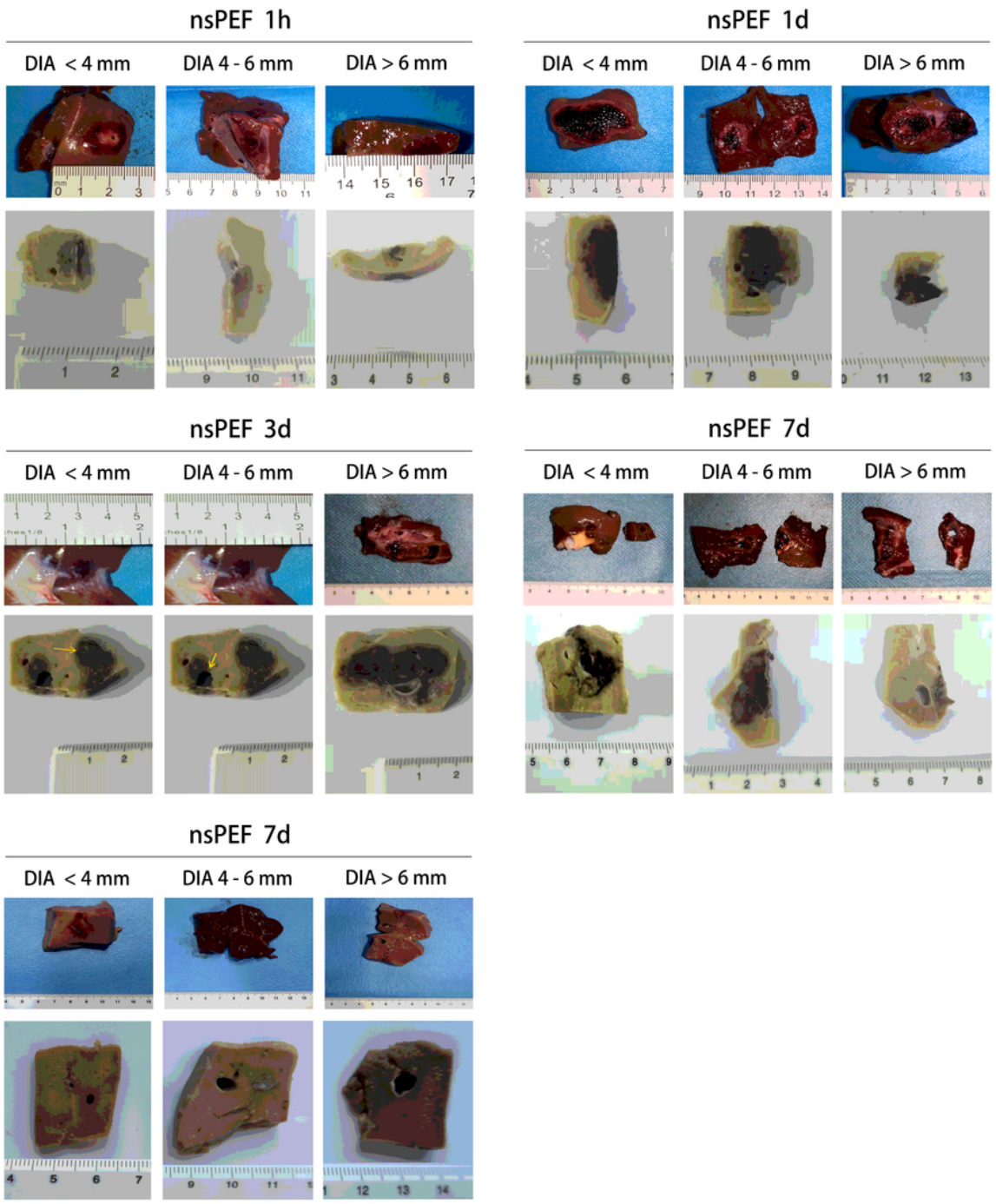


Fig. 3

Figure 3

Gross pathology of ablated porcine liver specimens. Figure 3 demonstrates the discoloration caused by ablation in the dissected liver and the well-defined boundaries of the ablated area on the formalin-fixed liver 1 hour, 1 day, 3 days, 7 days, and 14 days post treatment. Hepatic veins of different diameters are seen within the areas of ablation. The ablated area shows haemorrhagic change, with grossly intact hepatic structures and morphology 1 hour, 1 day, 3 days, and 7 days after treatment. After 14 days, the

haemorrhage had gradually resolved in the ablated area. A well-defined margin was visible between the ablated and non-ablated areas. The traversing hepatic veins of different diameters appeared intact within the ablated areas.

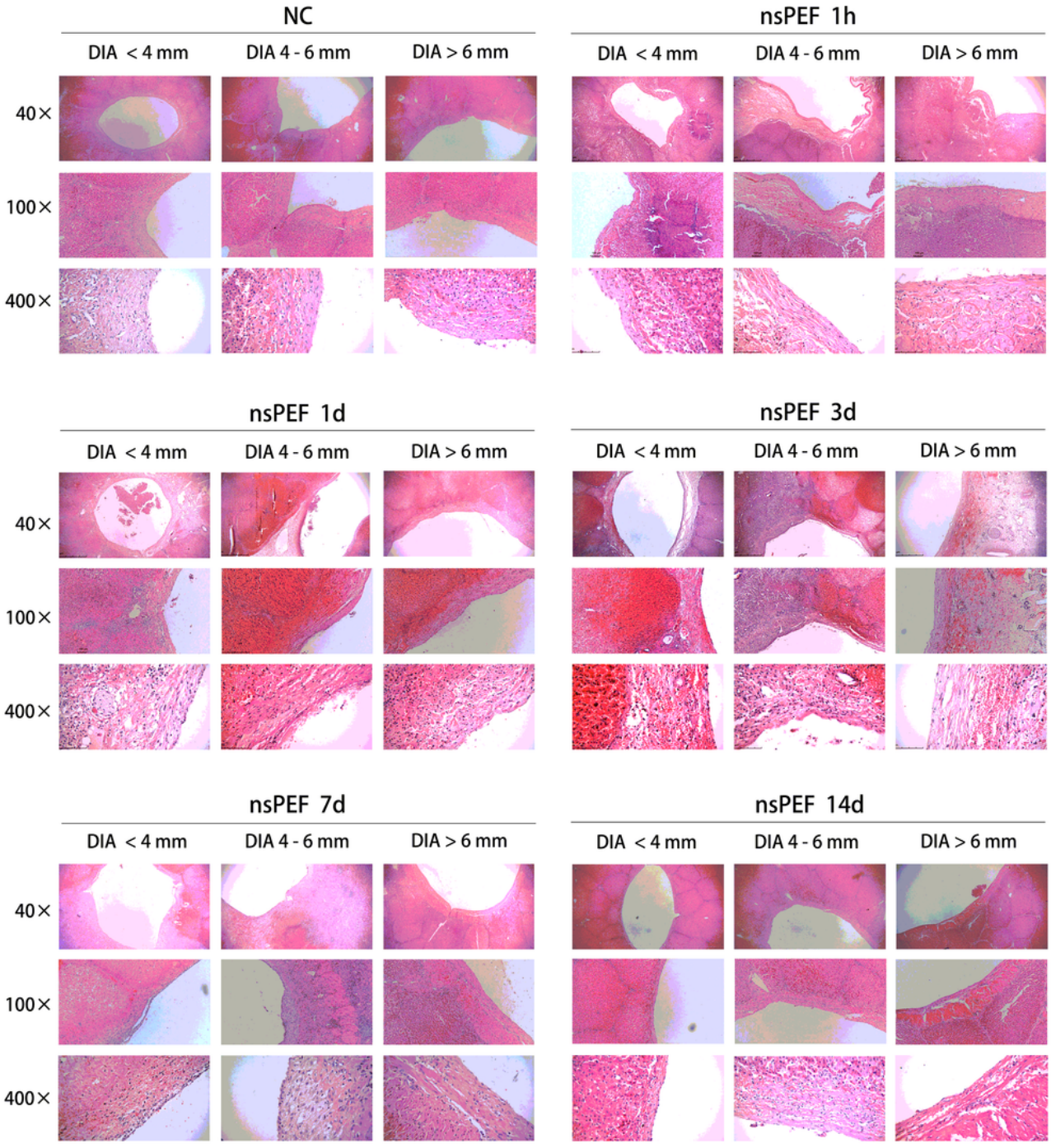


Fig. 4

Figure 4

H&E-stained sections. Figure 4 presents haemorrhagic changes and preservation of the hepatic vein wall, with relatively intact hepatic structures and morphology. The integrity of hepatic lobules was grossly

preserved, and their structure was visible and intact throughout the ablation area. The 1 hour, 1 day, 3 day, and 7 day H&E slides (especially those for day 1 and day 3) showed acute, extensive, and severe cell death. At 1 and 3 days, the ablated area was congested with neutrophils, and showed extensive eosinophil infiltration. Nevertheless, normal hepatic architecture was preserved. After 14 days, the ablated area appeared extensive hepatic regeneration and fibering. The hepatic veins of the three diameter groups (DIA<4mm: vascular diameter <4mm; DIA 4-6mm: vascular diameter 4-6mm; DIA >6mm: vascular diameter >6mm) demonstrated mild oedema without apparent endothelial and structural damage. H&E staining of the hepatic vein wall at 1 and 3 days post nsPEF show remarkable haemorrhagic congestion with red blood cells infiltration and residual inflammation in the subendothelial layer of the hepatic vein wall. The hepatic veins in the three groups appeared to be structurally preserved in the ablated area. Most importantly, relative preservation of normal vascular wall architecture, especially of the endothelial and smooth muscle layers, was observed.

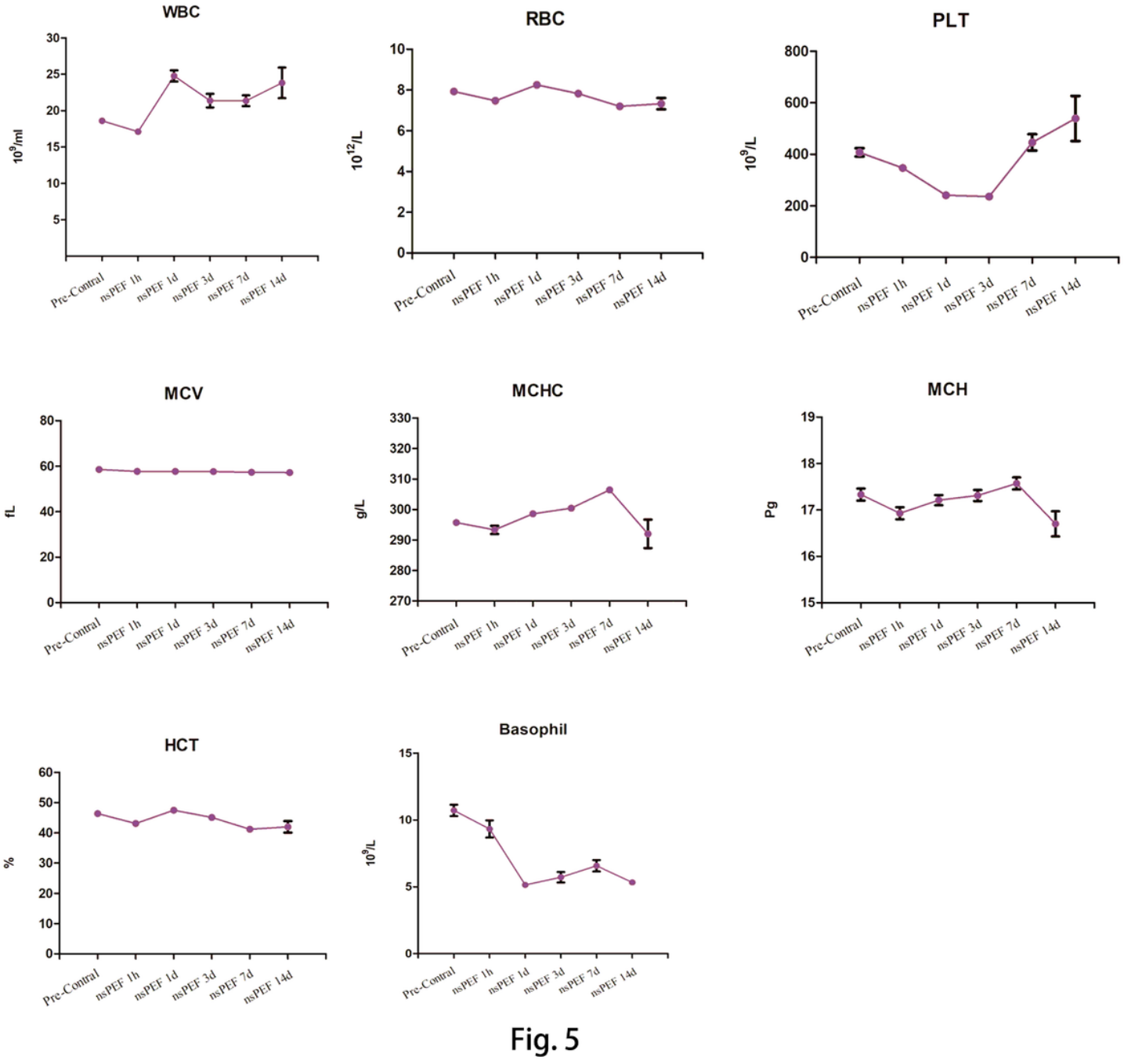


Fig. 5

Figure 5

Blood count tests. The blood tests included white blood cell (WBC), red blood cell (RBC,) and platelet (PLT) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haematocrit (HCT), and basophil count (BASO). The blood samples were collected at 1 hour pretreatment and at 1 hour, 1 day, 3 days, 7 days, and 14 days post treatment.

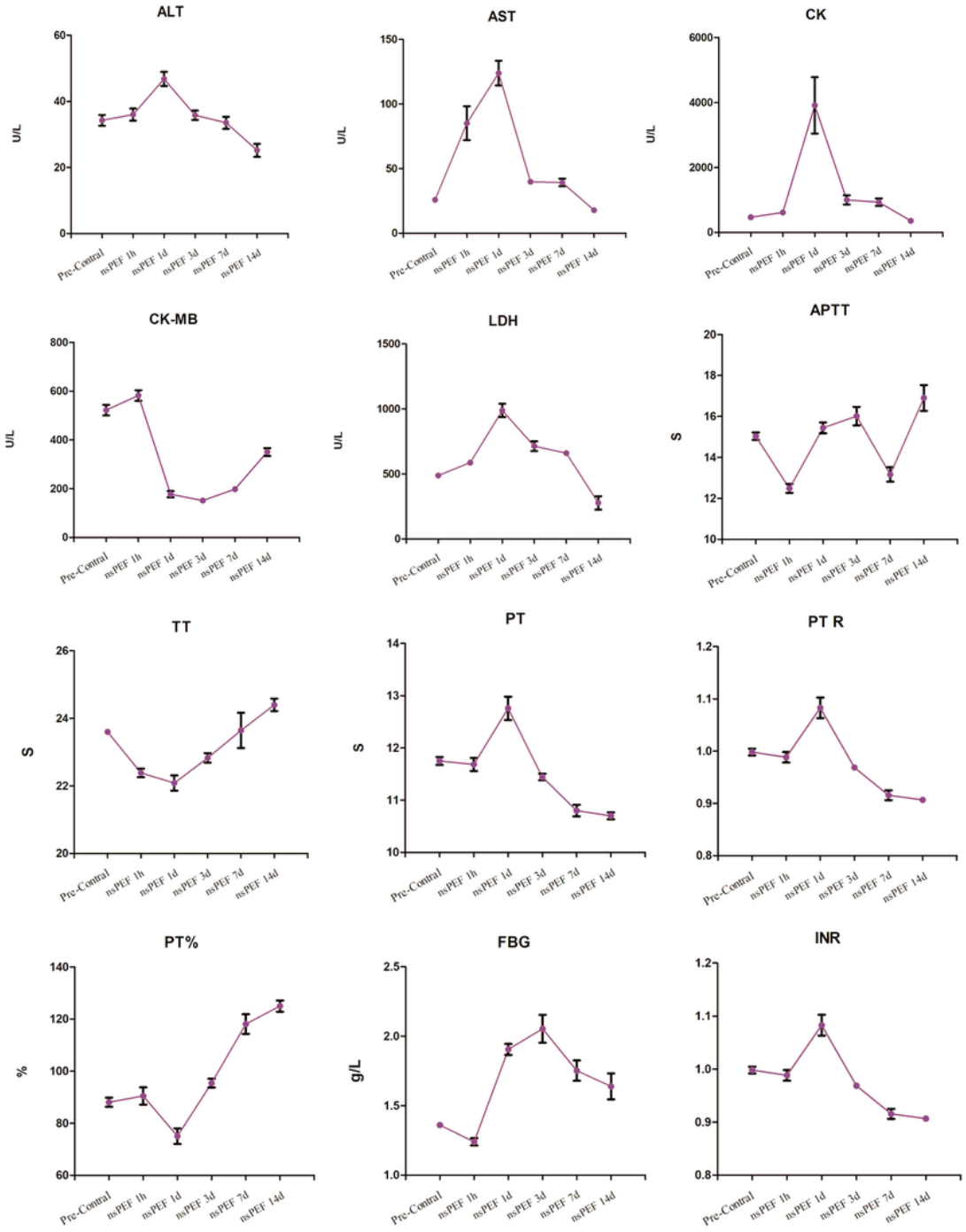


Fig. 6

Figure 6

Liver function, myocardial function, and coagulation function tests. Liver function was assessed by determining the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Myocardial function was assessed by determining the levels of creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH). Coagulation function was assessed by determining the activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), prothrombin

ratio (PT R), prothrombin time activity percentage (PT%), fibrinogen (FBG), and international normalized ratio (INR). The blood samples were collected at 1 hour pretreatment and at 1 hour, 1 day, 3 days, 7 days, and 14 days post treatment.