

Early Detection of Steroid-Induced Femoral Head Necrosis using ^{99m}Tc -Cys-Annexin V-based Apoptosis Imaging in a Rabbit Model

Xiaolong Wang

Inner Mongolia Medical University

Jianbo Li

Affiliated Hospital of Inner Mongolia Medical College

Da Man

Inner Mongolia Medical University

Rui Liu

Affiliated Hospital of Inner Mongolia Medical College

Jianmin Zhao (✉ nmzjmin@163.com)

Affiliated Hospital of Inner Mongolia Medical College <https://orcid.org/0000-0002-4201-2638>

Research article

Keywords: ^{99m}Tc -Cys-Annexin V, SPECT Imaging, MRI, Steroid-Induced Femoral Head Necrosis

Posted Date: September 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-68831/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on December 3rd, 2020. See the published version at <https://doi.org/10.1186/s10020-020-00248-1>.

Abstract

Background At present, the early diagnosis of femoral head necrosis mainly relies on MRI, and most early patients are difficult to make an accurate diagnosis. Therefore, to investigate the early diagnostic value of ^{99m}Tc -Cys-Annexin V SPECT imaging were compared with MRI in rabbit models of steroid-induced femoral head necrosis.

Methods The rabbit models of steroid-induced femoral head necrosis were established by intravenous injection of horse serum and gluteal muscle injection of methylprednisolone in of 5-month-old healthy New Zealand white rabbits. ^{99m}Tc -Cys-Annexin V SPECT imaging and MRI were performed at 2nd week, 4th week, and 6th week after modeling. After that, histopathology was used to verify the success of modeling. Apoptosis was detected by transmission electron microscopy and TUNEL.

Results At 2 weeks after the injection of hormone, ^{99m}Tc -Cys-Annexin V SPECT image showed abnormal radioactive uptake in the bilateral femoral head. And over time, the radioactivity concentration was more obvious, and the ratio of T/NT (target tissue/non-target tissues) was gradually increased. In the SPECT imaging at each time point, T/NT ratio of the model group was significantly higher than that of the control group ($P < 0.01$); at 4 weeks after the injection of hormone, MRI showed an abnormal signal of osteonecrosis. At 2, 4, and 6 weeks after hormone injection, apoptosis was observed by TUNEL and transmission electron microscopy.

Conclusion s ^{99m}Tc -Cys-Annexin V SPECT imaging can diagnose steroid-induced femoral head necrosis earlier than MRI, and has potential application value for non-invasively detecting early and even ultra-early stage of femoral head necrosis.

Full Text

This preprint is available for [download as a PDF](#).

Figures

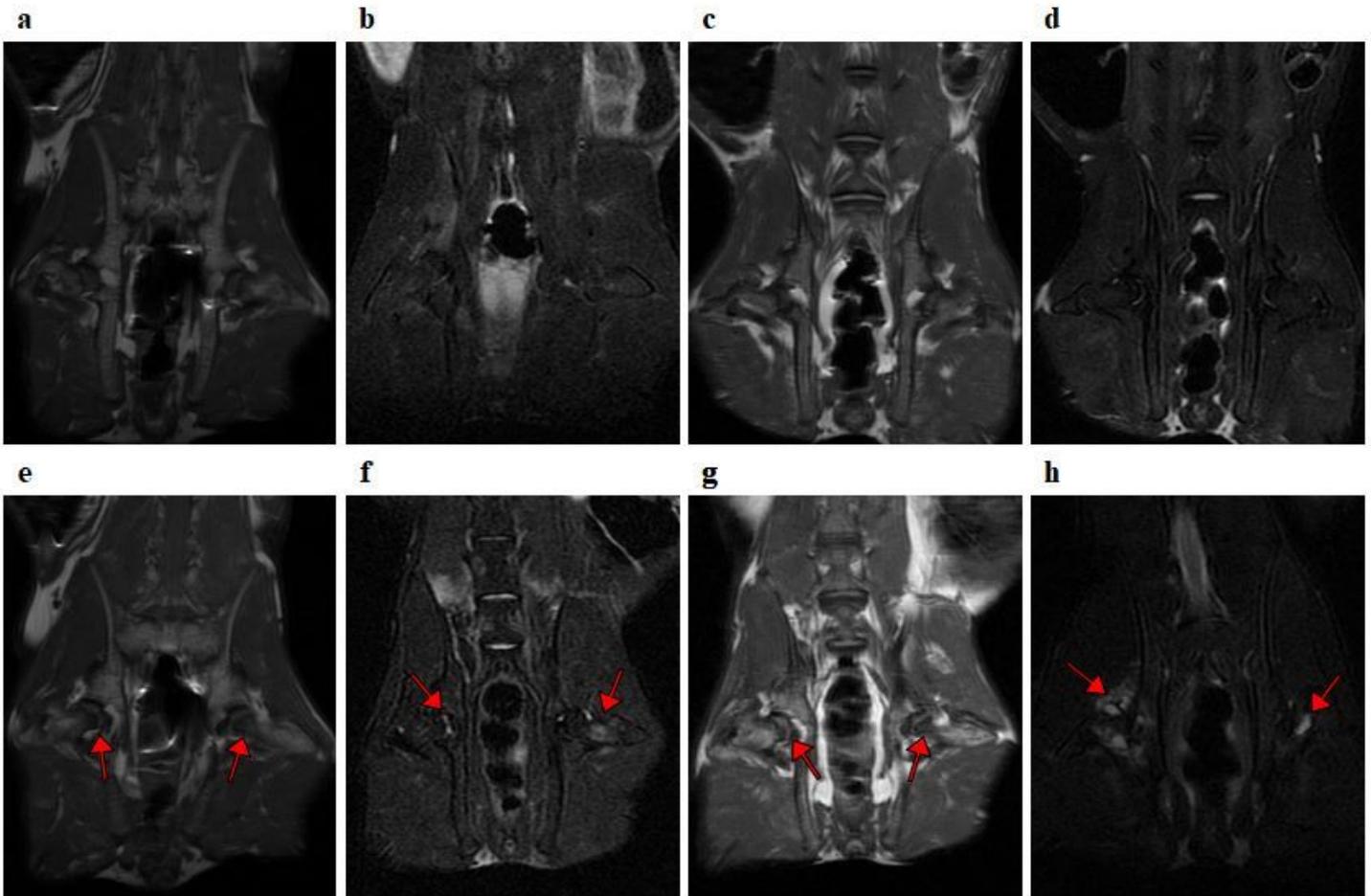


Figure 1

MRI Imaging. a and b were T1WI and T2WI + fat-suppression coronal image in the control group; c and d were T1WI and T2WI+ fat-suppressed coronal image at 2nd week after injection of hormone in the model group; e and f were T1WI and T2WI+ fat-suppressed coronal image at 4th week after injection of hormone in the model group; g and h were T1WI and T2WI+ fat-suppressed coronal image at 6th week after injection of hormone in the model group. (Red arrows indicated osteonecrosis slow/high signal in T1 WI and T2WI+fat suppression sequences)

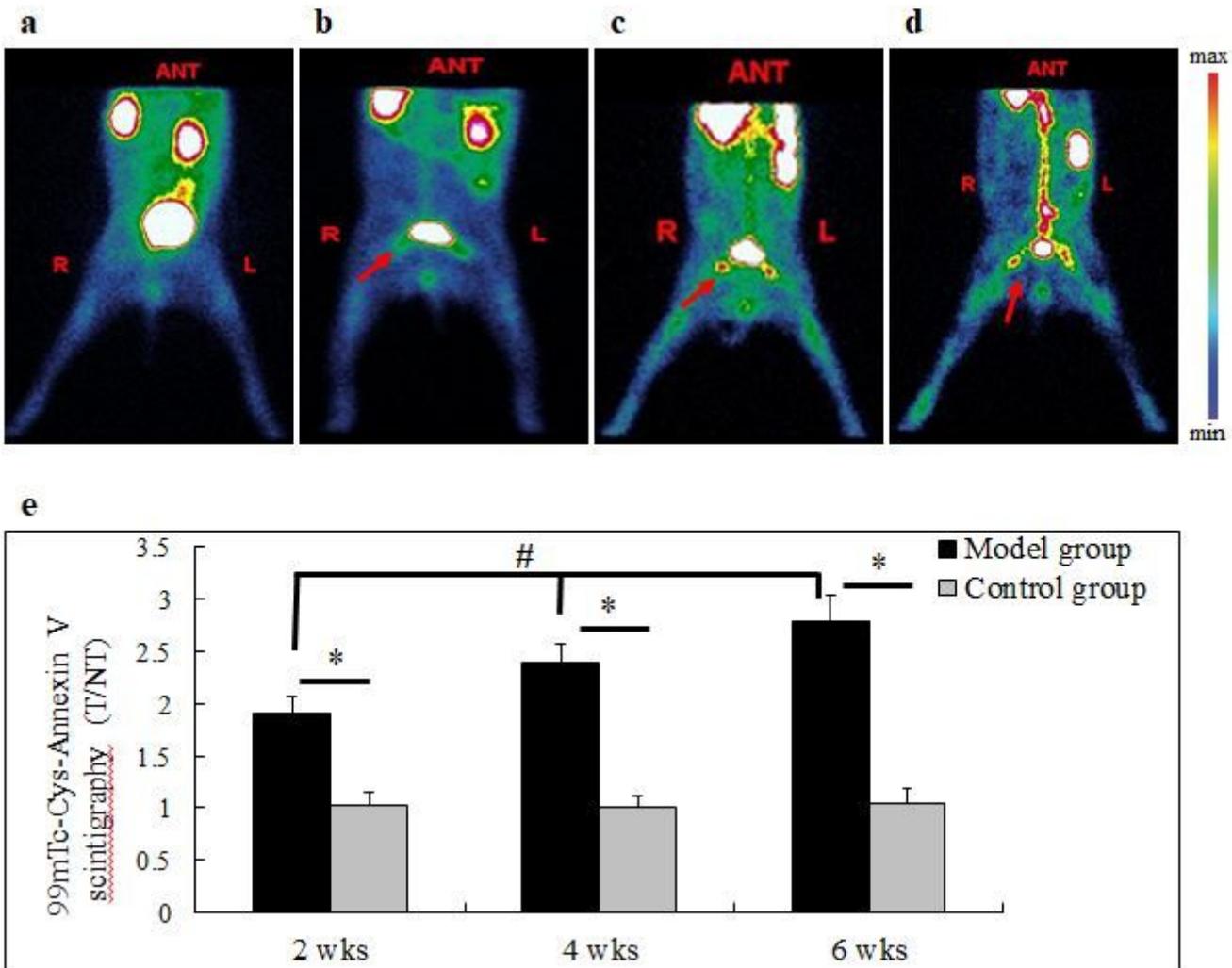


Figure 2

^{99m}Tc-Cys-Annexin VSPECT imaging. a was the control group image; b, c, and d were the images at 2nd, 4th, and 6th week after the injection of the hormone in the model group. (Red arrows indicated abnormal radioactive concentration in the bilateral femoral heads). e: The T/NT ratio of the model group was significantly higher than that of the control group at each time point (*inter-group comparison, $P < 0.05$), and there was a statistically significant difference in the T/NT ratio within the model groups at each time point (# intra-group comparison, $P < 0.05$). Model group vs control group: the 2nd week ($n = 9$ vs 8), 4th week ($n = 8$ vs 8), and 6th week ($n = 9$ vs 8).

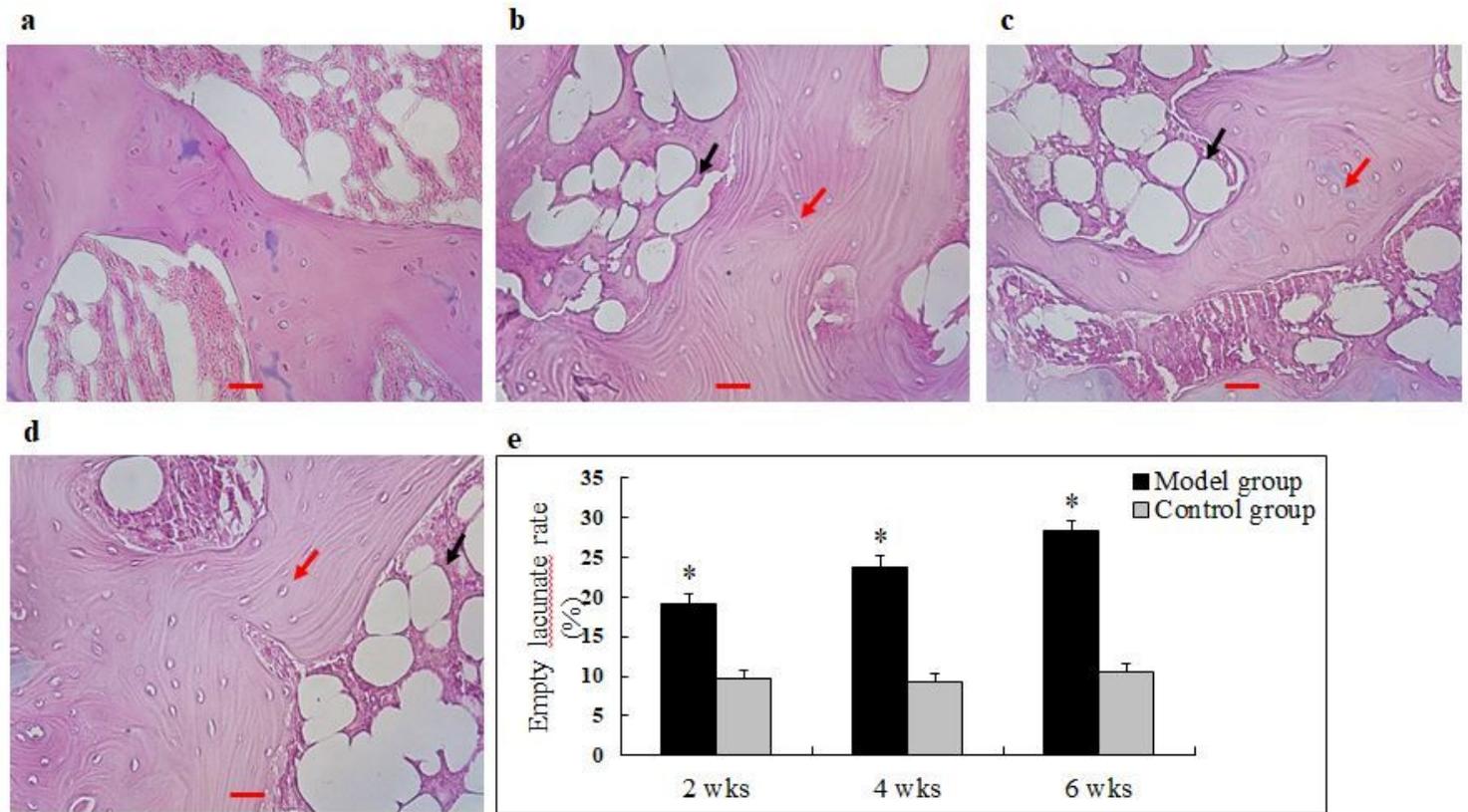


Figure 3

Histological images in bone tissues in both groups detected via H&E staining($\times 400$). No obvious osteonecrosis was observed in control group(a). Typical osteonecrosis of the femoral heads was observed in model group (b-d). Scale bar = 100 μm . The rate of empty lacunae in model group was significantly higher than that control group at each time point(e) (* compared with control group, $P < 0.05$). The red arrow shows the empty lacunae, and the black arrow shows the fat cells). Model group vs control group: the 2nd week (n= 9 vs 8), 4th week (n= 8 vs 8), and 6th week (n=9 vs 8).

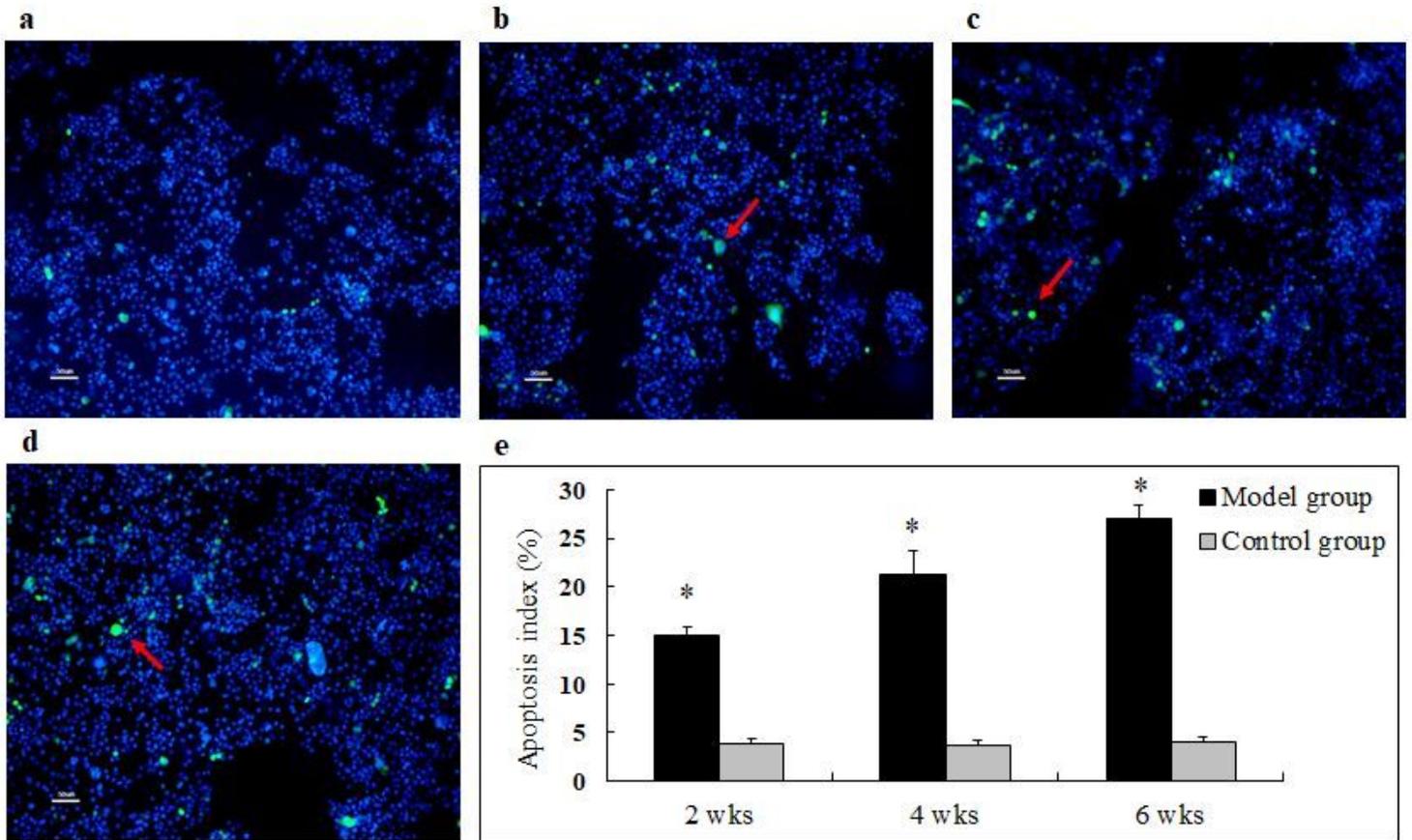


Figure 4

Apoptosis was detected via TUNEL assay with a fluorescence microscope ($\times 200$). There was no abnormal apoptosis detected in control group at any of the time points (images a). TUNEL staining showed gradual increases in apoptosis in model group (images b-d). Scale bar = 50 μ m. The apoptosis index in model group was significantly higher than that in control group at each time point (images e). DAPI was used to mark all cells, blue dots in the figure, and TUNEL was used to stain apoptotic cells, green dots in the figure) (* compared with control group, $P < 0.05$. The red arrow shows the TUNEL-positive cells). Model group vs control group: the 2nd week ($n=9$ vs 8), 4th week ($n=8$ vs 8), and 6th week ($n=9$ vs 8).

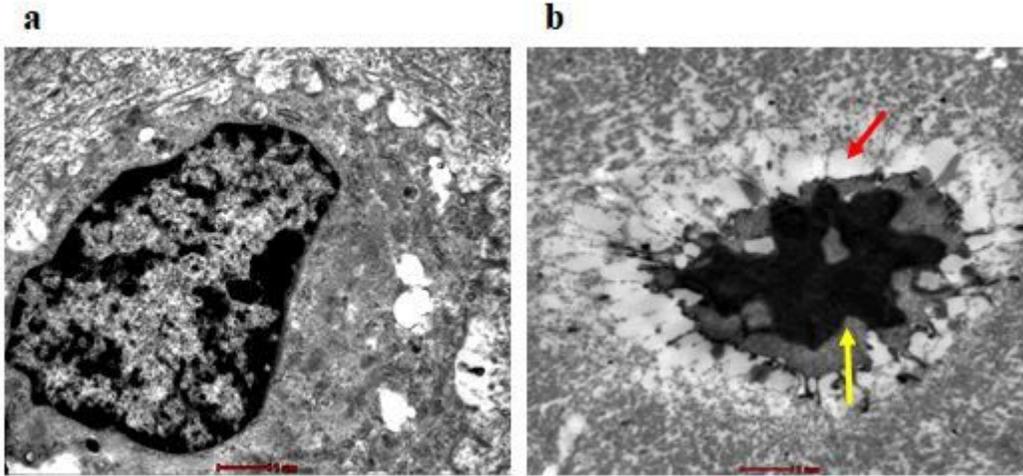


Figure 5

Morphological changes of apoptotic cells was detected via TEM ($\times 9900$). Control group (images a): Osteocyte was round or oval, large in size and rich in organelle, the chromatin was clear and uniform, and the nuclear membrane was intact. Model group showed typical apoptotic morphological features: the bone cell volume became smaller, the nucleus was pyknotic, the shape was irregular, the cell edge vacuolation, the nuclear chromatin was concentrated and marginalized (the red arrow), and the intracellular electron density was deepened, the heterochromatin was rich in the nucleus and distributed in large pieces (the black arrow). (images b). Scalebar=2 μ m.

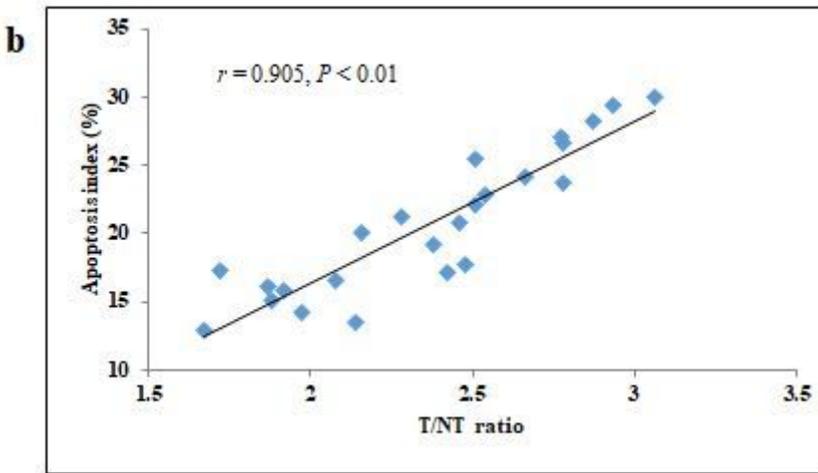
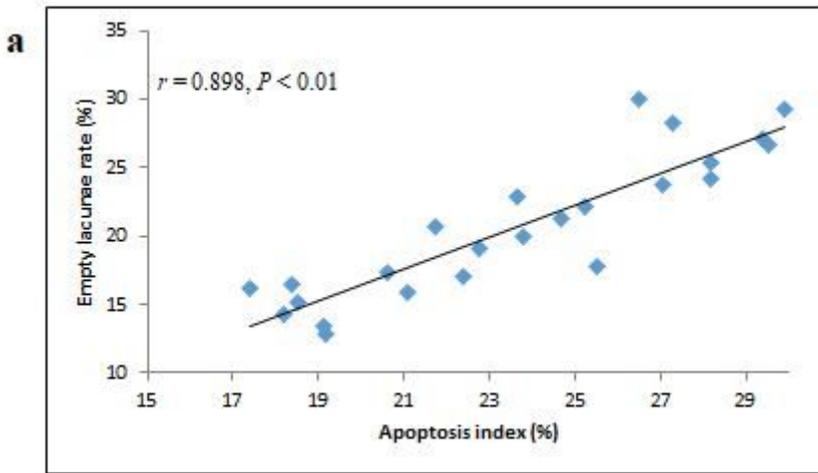


Figure 6

Pearson correlation analysis. Positive correlation between the empty lacunae rate and the apoptosis index (a), positive correlation analysis between the apoptosis index and the T/NT ratio (b). T/NT, target tissue and non-target tissue.