

Impact of PVA coated Nanoparticles on cellular viability and functionality of immune cells obtained from healthy donors and patients with rheumatoid arthritis or osteoarthritis

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Background: Nanotechnology has developed into a key technology of the 21st century. Over the recent years, the number of nanotechnical products has received an enormous boost. More and more efforts are currently being done to use this technology also in rheumatology for diagnostic and therapeutic purposes (see www.nanodiara.eu). Therefore, crucial questions concern the safety aspects. Thus, the focus of our work here was to identify putative effects of nanoparticles on human immune cell function.

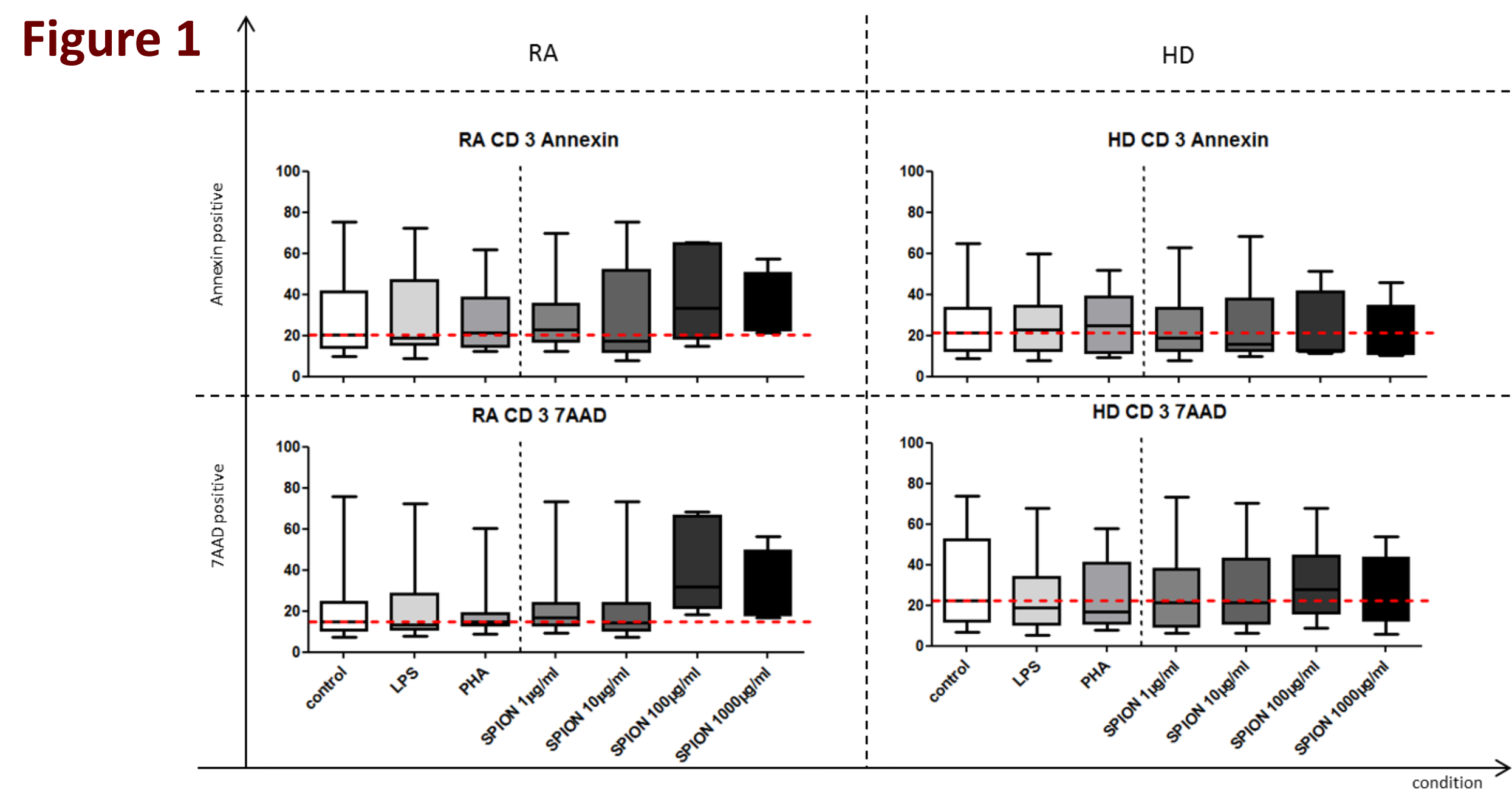


Figure 1: Influence of amino PVA-SPIONs on the survival of human immune cells. Whole blood survival analysis was performed with blood samples obtained from patients suffering from RA (left) or healthy donors (right). Cells were analyzed for specific surface markers (e.g. CD3), Annexin V (apoptosis marker) and 7AAD (necrosis marker).

Objectives: We analyzed clinical relevant interactions between polyvinyl alcohol (PVA) coated super paramagnetic iron oxide nanoparticles (SPIONs) and human immune cells in the presence or absence of dexamethasone.

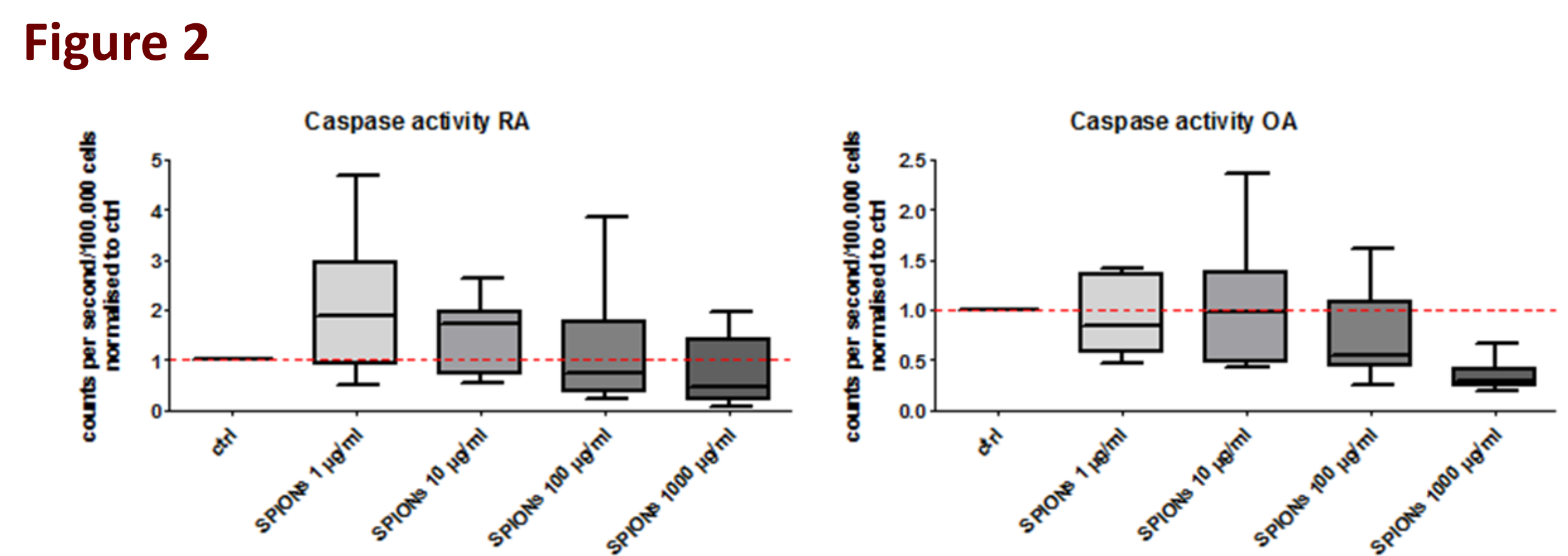


Figure 2: Impact of amino PVA-SPIONs on the caspase activity of human T cells obtained from RA (left) and OA (right) patients. Caspase measurements were performed to investigate cellular toxicity of amino-PVA-SPIONs on human T cells.

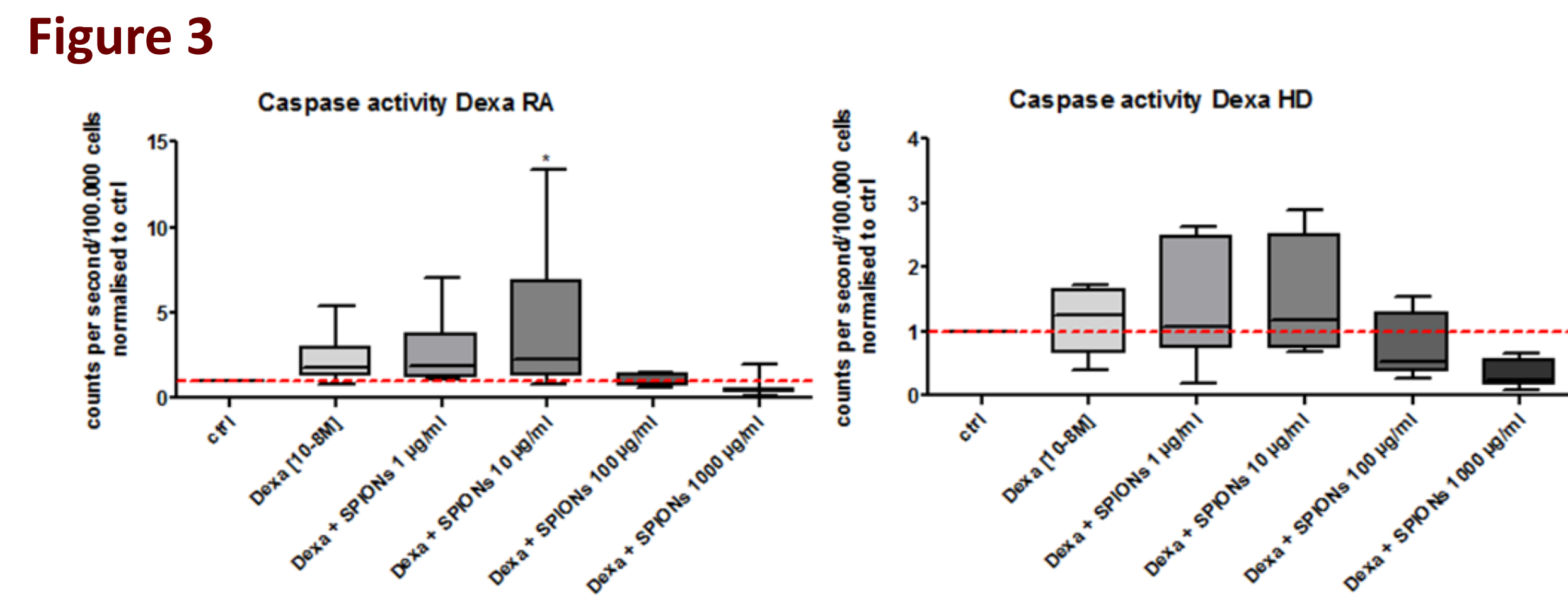


Figure 3: Impact of amino PVA-SPIONs in the presence of dexamethasone on the caspase activity of human T cells obtained from RA patients (left) and healthy donor (right). Caspase measurements were performed to investigate cellular toxicity of amino-PVA-SPIONs on human T cells.

Methods: 100µl venous blood (obtained from healthy donors or patients suffering from RA) was diluted with 100µl serum free medium. Cells were stimulated with amino PVA-SPIONs (1µg/ml, 10µg/ml, 100µg/ml, 1000µg/ml), the according controls (not shown) or left untreated for 20h. After lysis of erythrocytes, cells were stained for extracellular markers (e.g. CD3), Annexin V-PE/7-AAD and analyzed by flow cytometry. PBMCs were isolated from blood obtained from healthy donors, RA and OA patients and CD4 positive T cells were separated via MACS-sort. Cells were incubated in medium with/without dexamethasone treatment at the clinical relevant concentration 10⁻⁸M. Amino PVA-SPIONs were added at varying concentrations and cells were incubated for 24h. Apoptosis was analysed by measuring the caspase-3/7-activity. Caspase-3 and -7 are members of the cysteine aspartic acid-specific protease family, which play a key effector role in apoptosis in mammalian cells. Furthermore, CD4 positive T cells were incubated with/without PHA, with/without 10⁻⁸M dexamethasone and/or with/without PVA-SPIONs at different concentrations. Functionality was determined via proliferation measurements of CFSE labeled T cells after 72h under normoxic (5% CO₂ and 18% O₂) or hypoxic (5% CO₂ and <1% O₂) conditions by flow cytometry.

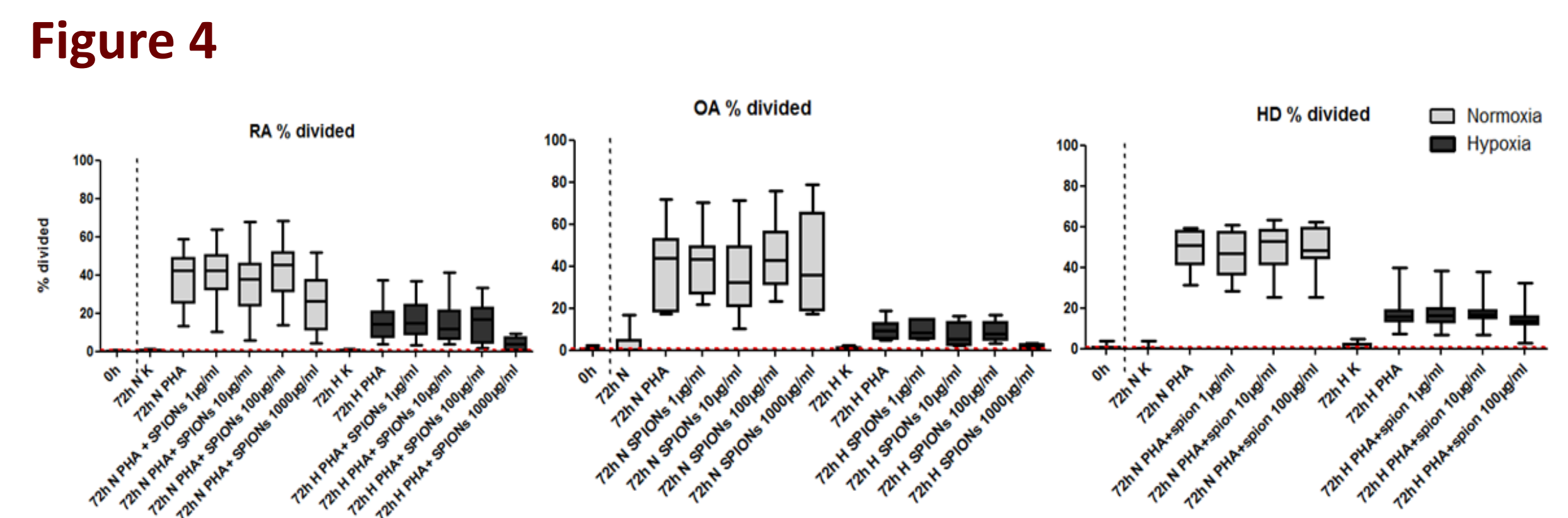


Figure 3: Proliferation of T cells from RA (left) and OA (middle) patients and healthy donors (right) treated with varying SPION concentrations (1-1000 µg/ml). SPIONs did not show an effect on the proliferation of T cells under normoxic or hypoxic conditions.

Results: Survival assay revealed that amino PVA-SPIONs do not affect the survival of human T cells within the whole blood (figure 1). Similar results were found for isolated T cells: Caspase measurements to investigate cellular toxicity of amino-PVA-SPIONs did not show any measurable effects on the survival at concentrations less than 1000µg/ml (figure 2). Interestingly, SPION treatment with increasing concentrations in the presence of dexamethasone even resulted in a decrease of caspase activity indicating a diminished apoptosis of the T cells in RA patients as well as in healthy donors (figure 3). Dexamethasone itself did not have any effects on caspase activity. As expected, there was less proliferation under hypoxia than under normoxia (figure 4); and treatment with dexamethasone decreased the percentage of divided cells for both RA patients and HD under normoxia and hypoxia (figure 5). Focusing on the influence of dexamethasone on the T cell proliferation in the presence of PVA-SPIONs, we observed no difference in the impact of dexamethasone on proliferation (figure 5).

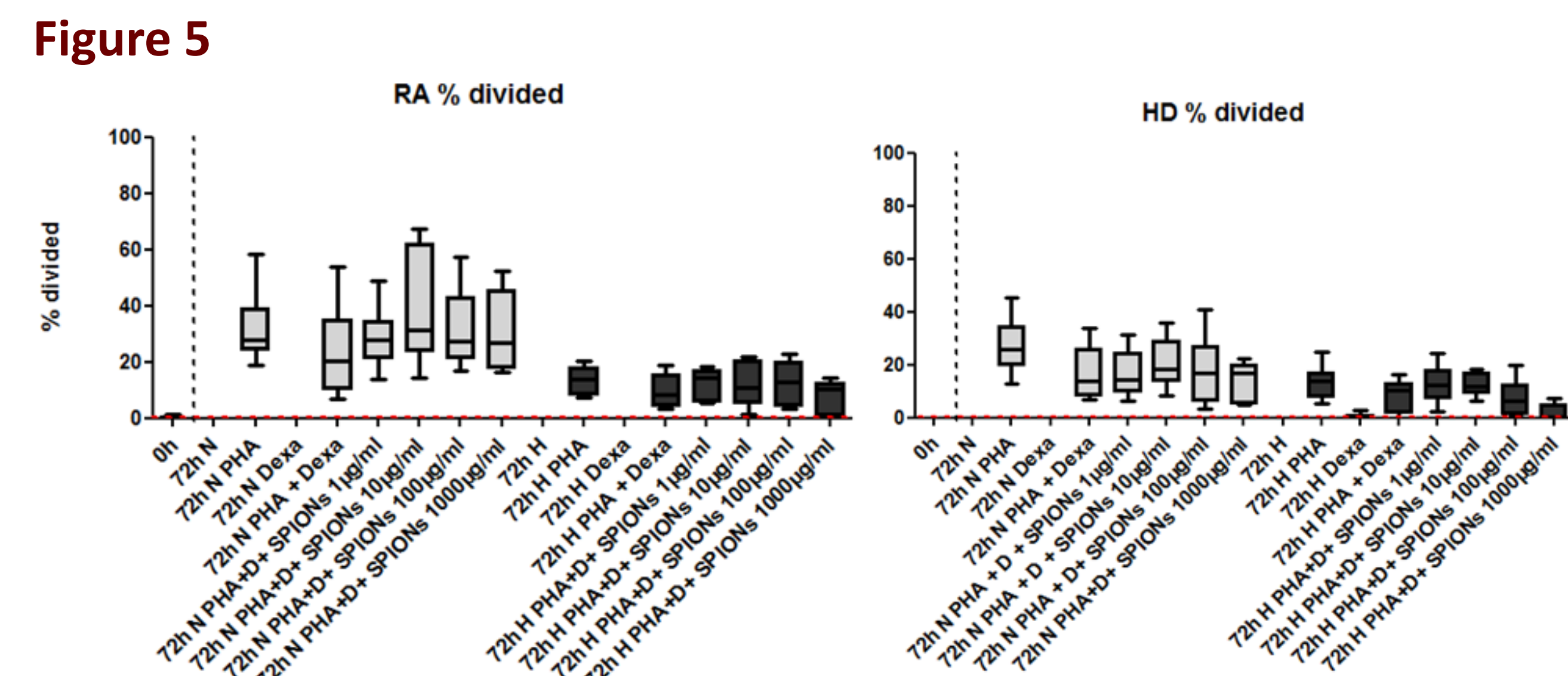


Figure 5: Proliferation of T cells obtained from RA patients (left) and healthy donors (right) in the presence of dexamethasone and treated with varying SPION concentrations (1-1000 µg/ml). SPIONs did not affect the proliferation of T cells under normoxic or hypoxic conditions.

Conclusion: PVA coated nanoparticles at concentrations up to 1000µg/ml do not interfere with effects of dexamethasone on proliferation and caspase-3/7-activity of human T cells. The impact of PVA-SPIONs on other human immune cells and on effects of glucocorticoids on these cells need to be further analyzed. This represents a critical need prior to the clinical use of nanoparticles in rheumatology.

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