How to plan capillary evaluation on small biopsy samples?

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Introduction

Conflicting results in literature about capillary supply of muscles and muscle fibres encouraged us to develop methods for 3D evaluation of capillaries in skeletal muscles, such as slicer, tracer, and automatic segmentation of capillaries, combined with manual correction in virtual reality. Though time-consuming, the estimation error of these methods proved to be essentially lower and the amount of usefull information thus obtained is higher compared to traditional counting of capillary profiles in 2D sections. Compared to rat muscles, more anastomoses exist among capillaries in human muscles and consequently, capillary profiles in 2D sections are round, oval and longitudinal (see Fig.1 middle image).The aim of our studies was to establish protocol for analysis of capillary supply in biopsy samples, especially from small needle and forceps biopsies (see Fig. 1 left). Oblique and longitudinal profiles of muscle fibres are usually present in such biopsies, since it is difficult to orient the tissue appropriately. The samples are small and even with well-trained technicians it is impossible to avoid artifacts resulting from cutting. It is also difficult to follow structures on successive physical sections.

Methods

Our methods were tested on autopsy samples of human vastus lateralis and masseter muscles, the latter were compared in young and old subjects.The results were expressed as the length of capillaries per unit volume of muscle tissue (Lcap/Vmuscle), per fibre volume (Lcap/Vfib), fibre surface area (Lcap/Sfib), per fibre length (Lcap/Lfib); further, we measured average number of branches per muscle volume (Nbr/Vmuscle), the average length of a capillary branch (Lbr), tortuosity and anisotropy. In the masseter muscle capillarization of pure type I fibres was compared with pure type II fibres.

Results and discussion

It is well known that skeletal muscles consist of pure and hybrid fibre types which is connected with the prevailing metabolism as well as contractile features of muscle fibres. When speaking of capillary supply per fibre, it concerns an average fibre type within a muscle. In human muscles we found Lbr as the most stable parameter which did not differ among vastus lateralis, multifidus and masseter muscles. Lcap/Vmuscle and Lcap/Vfib were much higher in masseter compared to multifidus. Within masseter muscles Lcap/Lfib was much higher in type I fibres in young compared to old subjects.

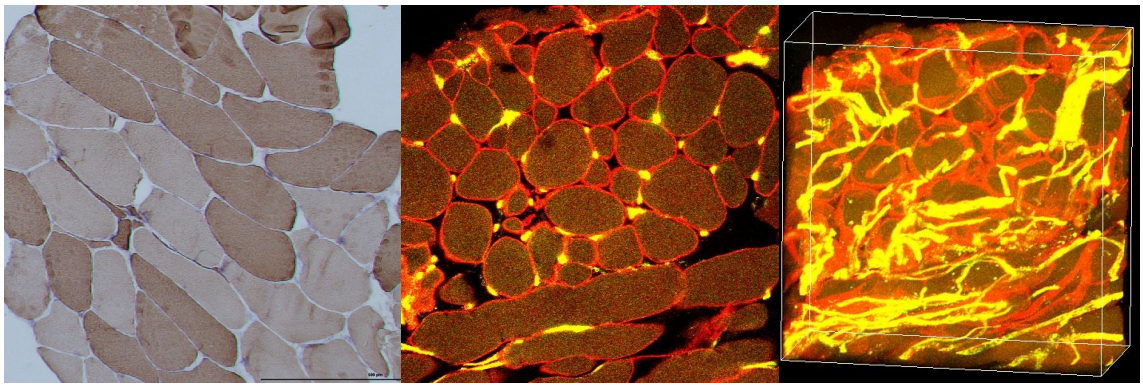

Fig.1. Muscle fibres in vastus lateralis muscle (left), muscle fibres and capillaries in masseter (middle), and volume rendering of capillaries and muscle fibres from the middle image (right) . Left -forceps biopsy, middle and right - autopsy samples.

How can we use this knowledge to make an efficient and economic capillary analysis in small biopsies, where the material at disposal is limited. Sampling itself is questionable. 2D analysis is in principle possible around a defined fibre type, however, capillary profiles are counted in a single thin cross-section which might result in an extremely high estimation error. 3D analysis on the other hand has some advantages, as it encompasses larger tissue volume and enables 3D reconstruction of capillaries supplying muscle fibres.

Conclusion

Although not ideal and for sure also error prone, 3D analysis provides more information. Further analysis will probably give the answer whether capillarization in the limited biopsy sample is reflected better by Lcap/Vmuscle or Lcap/Vfib.

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