TEAR FILM BIOMARKERS AS PROGNOSTIC INDICATORS FOR RECURRENT PTERYGIUM

ZAKARIA N., DE GROOT V., TASSIGNON M.J.

Affiliation Institution: Department of Ophthalmology, Antwerp University Hospital

Promoters of the Project: DE GROOT V., MD, PhD TASSIGNON M.J., MD, PhD

BACKGROUND AND AIM OF THE PROJECT

Pterygia are inflammatory fibrovascular wing shaped lesions that invade the cornea. They are characterized by proliferation of fibrovascular tissue encroaching onto the cornea which may be sight threatening due to astigmatism, tear film disturbances and/or occlusion of the visual axis. There is much debate surround the pathogenesis of pterygium and a number of theories have been put forward including genetic instability, cellular proliferation, inflammatory influence, degeneration of connective tissue, angiogenesis, aberrant apoptosis or wound healing process and stem cell dysfunction. Treatment of pterygia entails its surgical excision, however in some cases they aggressively recur. This led us to investigate the role of biomarkers for early detection of recurrent pterygium after surgical resection.

The aim of this project is to establish the use of IL-6, IL-8 and VEGF as biomarkers in the tear film for early detection of recurrent pterygium.

The reason why we chose IL-8 as a possible biomarker for pterygium is because it is a product of activated monocytes, fibroblast, endothelial and epithelial cells capable of inducing the production of matrix metalloproteinases (MMPs) which are enzymes shown to be localized at the advancing edges of the pterygium (1). Similarly, IL-6 synthesized by fibroblasts, endothelial cells and keratinocytes can also induce the expression of MMPs. VEGF or vascular endothelial growth factor has been demonstrated to have a chemotactic effect on endothelial progenitor cells and has been reported to be expressed in pterygial epithelium (2).

DEVELOPMENT OF THE PROJECT

Looking at the data from our previous CBA cytokine study that took into account IL-6, IL-8, VEGF, MCP1 and FasL levels in patients with vascularized corneas [bacterial keratitis (n=1), limbal stem cell deficiency (n=5), keratoconus (n=1), chemical burn (n=3) and aniridia (n=2)] we showed significantly higher levels of IL-6 (p=0.04), IL-8 (p=0.03) and VEGF (p=0.03) in the corneal epithelial secretions of vascularized corneas compared to normal control eyes. Since statistically significant results were only obtained in the corneal epithelial secretion samples compared to basal or reflex tears, we can assume that this is because of local production of the cytokines. Hence we conclude that this would be the best method for obtaining our samples in the present study.

Following ethical committee approval and proper informed consent, patients having pterygium in one or both eyes will be enrolled into the study. Ocular surface photographs will be taken and the pterygium will be graded according to the comprehensive system for pterygium classification put forward by Johnston, Williams & Sheppard (3). Following one or two drops of a

local anesthetic, a large silicon rubber cornea bath (encompassing the cornea and limbus) will be applied to the eye and two drops of saline instilled. The epithelial secretions diluted with the saline will be immediately picked up using a micropipette and all samples stored in cryovials at -80°C until analysis. Following surgical resection of the pterygium, pterygia tissue will be sent for immunohistochemical analysis for CD43, SCD5 and MEFV (all these genes are upregulated at least 2-fold in recurrent pterygia compared to primary pterygia). Epithelial secretions and ocular surface photographs will be collected again at 2 weeks, 3 month and 1 year post op.

Age and gender matched volunteers having no prior history of eye disease, dry eyes or contact lens wear, will be selected as the control group to determine the normal baseline cytokine/growth factor levels. The time of tear collection will be fixed in all subjects to 12:00 pm to avoid diurnal variation of cytokine levels.

A standard capture sandwich assay will be used to determine the cytokine levels in tears. Each captured antibody is coupled to a different set of beads which are internally dyed to express different levels of fluorescence thereby assigning it a specific spectral address. Each set of beads is coupled to a monoclonal antibody against the specific cytokine or growth factor. The diluted standards or the tears are added to the Ab-bead reagent and Ab-phycoerythrin (PE) detector reagent and incubated for 3 hours at room temperature. Thereafter the beads are washed to remove any unbound detector antibody-PE reagent before data acquisition using a BD FACS Array flow cytometer. The data can then be analyzed using BD cytometric bead array software. Once the absolute concentrations of the cytokines have been determined, statistical analyses can then be carried out to determine the difference in levels of cytokines in the study group versus that of the control group.

By establishing higher tear film levels of IL-6, IL-8 and VEGF in eyes with pterygia compared to normal eyes and the return to baseline levels post excision, we can begin to ascertain the role of these key players in the pathogenesis of pterygia. But if we can also show high levels of these cytokines/growth factors in subjects that present with recurrence post excision, we will be able to quantitatively as well as qualitatively justify the role of IL-6, IL-8 and VEGF as prognostic indicators of recurrent pterygium.

REFERENCES

