Computerized in vivo classification of methylene blue stained fallopian tube mucosal damage: preliminary results

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Summary

Objective: Fertiloscopy is a simple minimal invasive method which allows salpingoscopy and microsalpingoscopy in order to examine the mucosa of the fallopian tubes of patients with unexplained infertility. Infectious tubal damage is a common cause of tubal infertility. In 1998 it was demonstrated that nuclear staining of cellular nuclei during microsalpingoscopy with methylene blue provides a simple in vivo method to evaluate cellular damage of the tubal epithelium. The purpose of this study was to introduce and statistically test a new computerized method to objectively evaluate the extent of tubal damage. *Design of Retrospective Study:* Cooperation of two Departments of Gynecology and Obstetrics (Krankenanstalt Rudolfstiftung, Vienna, Austria and CRES Center, Hôpital Natecia, Lyon, France) with the University of Art and Design, Linz, Austria and University Hospital, Vienna, Austria. *Materials and Methods:* Microsalpingoscopic images from ten female patients, aged between 18 and 45 years with primary infertility, showing stained nuclei in damaged intrafallopian tubal epithelium were provided by Antoine Watrelot, CRES Center, Hôpital Natecia, Lyon, France. These images were evaluated by an experienced medical expert staff examiner and a computerized standard method called cross-correlation and template matching. The obtained numbers of nuclear stainings were statistically evaluated. *Results:* Computerized evaluation of nuclear staining of damaged intrafallopian epithelial cells in female patients with infertility obtains similar but more reproducible results compared to manual evaluation ($p = 0.007$). *Conclusion:* Normalized cross-correlation can be used to measure tubal damage diagnosed by in vivo methylene blue dyeing during microsalpingoscopy and might facilitate the decision for in vitro fertilisation in patients with unclear unexplained infertility in further studies.

Key words: Infertility; Classification; Image processing; Fertiloscopy; Microsalpingoscopy; Diagnostic Tool; Methylene blue.

Introduction

Hysterosalpingography (HSG) is often used as a diagnostic tool in the workup of female infertility, but studies [1, 2] show low detection rates for tubal pathologies in respect of peritubal adhesions and the grade of inflammatory damaged tubal mucosa. Extrinsic infectious tubal damage, often induced by Chlamydia trachomatis, Neisseria gonorrhoea or multibacterial infections represent a common cause of tubal infertility or repeated tubal pregnancies [1, 3]. Until now most of the research with infectious agents has been conducted in epithelial cell lines (e.g. Chlamydia trachomatis in HEp-2 cells and HeLa-229) or in mice [4].

However, greater attention needs to be paid to methods for characterizing infectious in vivo changes to detect tubal infertility or prevent ectopic intrafallopian pregnancies.

Marconi and Quintana demonstrated in 1998 that dye staining of cellular nuclei during microsalpingoscopy with methylene blue provides a simple in vivo method to evaluate cellular damage of the tubal epithelium. Besides the presence of adhesions, it was mainly the dye-stained nuclei of damaged cells in the fallopian tubal mucosa which showed a high correlation with infertility rates [2].

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Compared to fertiloscopy, laparoscopy provides another diagnostic and therapeutic possibility to examine unexplained infertility [5]. However, fertiloscopy is an easier and less invasive procedure [6]. During the last decades, fertiloscopy has been established and has enabled microsalpingoscopy and tubal reconstructive therapy by transvaginal pelvic endoscopy [7, 8].

The object of the present study was to design a simple computerized image processing system of methylene blue stained tubal areas obtained by fertiloscopy to allow a uniform and objective classification of intratubal mucosal damage.

Materials and Methods

Chromopertubation is a standard procedure during laparoscopy and fertiloscopy and is associated with minimal complications. [9] For microsalpingoscopy under fertiloscopy, a rigid endoscope (2.9 mm diameter, with 30 degree lens) type: 26120 BHA, was used. By increasing the magnifying power to 60-fold, nuclear dye staining in damaged tubal epithelial cells can be observed. All fertiloscopy procedures were performed by using two special single-use trocars.

Images from ten patients with stained nuclei in damaged intrafallopian tubal epithelium were provided by Antoine Watrelot, CRES Center, Hôpital Natecia, Lyon, France. Cell nuclei in examined tubal epithelium were stained with 20 ml of methylene blue

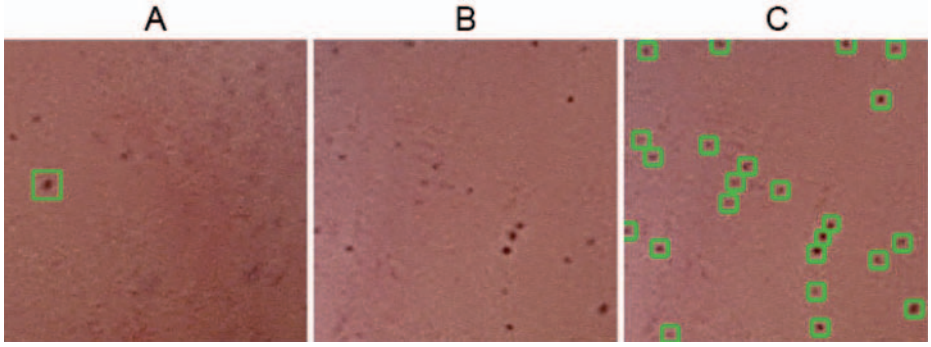

Figure 1. — The left image (A) shows an unprocessed image sample of a fallopian tube mucosa with methylene blue nuclear staining in vivo, in 60-fold magnification. The green rectangle highlights a single stained cell which is used as search template for the center image (B). The search result is illustrated in the image on the right (C). All occurrences of a stained cell are highlighted with a green rectangle.

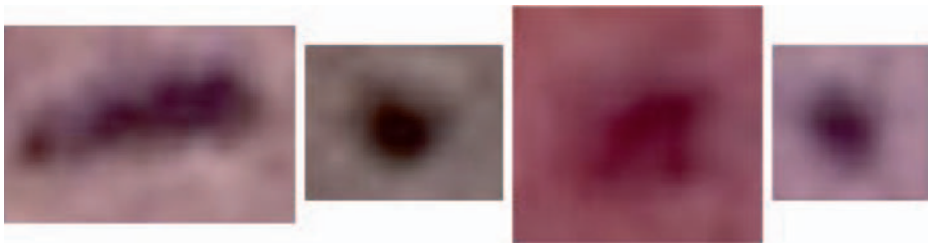

Figure 2. — The four images illustrate the criteria the authors defined for selecting the templates on the fallopian tube mucosa. Blue colored spots with sharp margin, round or oval-shaped, and with homogenous staining were counted in the pictures of inflamed fallopian tube mucosa.

dissolved in saline solution (NaCl 10%) by injection through a cervical cannula during fertiloscopy. For better visibility and to wash away excessive dye, saline solution was insufflated through the irrigation sheath of the endoscope used to perform salpingoscopy [2].

These digital color images were then processed with a software the authors developed for methylene blue stained cell recognition. The implemented method was based on template matching, which is a standard digital image processing [10] method to localize a known template in unknown samples. In the present application, the known template was an image clip of a single blue stained cell, which represented typical characteristics of an infectious tubal damage (shape, color, brightness, color of the area surrounding the cell with nuclear staining), as shown in Figure 1.

The authors' software localizes occurrences of several predefined templates, illustrated in Figure 2, and counts the number in all images per subject. The number of detected methylene blue stained cells was then compared with the number of cells counted by an experienced medical expert staff examiner.

Sharp margined and homogenous stained cells were selected as templates to be counted. Predefined templates used in the present matching procedure in this classification system are shown in Figure 2.

Since the processed images were collected in the prior ten years, informed consent for our retrospective computerized evaluation of the microscopic images sampled during fertiloscopy procedures was not obtained.

For statistical analysis, these images were each evaluated manually (by eye) and under computerized analysis, two times. For each patient, the arithmetic mean and the absolute value of the difference of the two computerized and the two manual counts were calculated. A Pearson correlation coefficient between these mean values and a Wilcoxon signed-rank test for the obtained differences were then performed. Results were considered statistically significant at two-tailed p values < 0.05.

Template matching

Template matching is a standard digital image processing method [10] to localize a known template in unknown samples. The method is used for counting the number of occurrences of several predefined templates in a series of image samples per subject. Since the brightness varies across sample images due to the photo-optical conditions of different camera systems, a normalized cross-correlation (NCC) function as a measure of match is applied. The NCC is calculated for each pixel in the sample image i with size M x N according to the template t with size U x V:

$$NCC(x,y) = \frac{\sum_u \sum_v [i(x+u,y+v)-\bar{i}][t(u,v)-\bar{t}]}{\sqrt{\sum_u \sum_v [i(x+u,y+v)-\bar{i}]^2 \sum_u \sum_v [t(u,v)-\bar{t}]^2}}$$

whereby x = 0, 1, 2, ..., M–1, y = 0, 1, 2, ..., N–1, u = 0, 1, 2, …, U-1, v = 0,1,2,…,V-1, $\bar{t}$ represents the average of t and $\bar{i}$ represents the average of i at the current location of t [11]. Figure 3 illustrates the NCC result for a given sample and its template image.

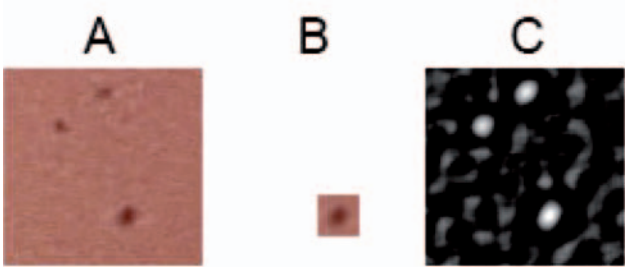

Figure 3. — The left image (A) shows the unprocessed sample with three blue stained cells. The center images is the template image to search for in image A. Image C shows the NCC result, which denotes the similarity with the template at the position of each pixel in the sample image. Bright regions indicate a high probability for a template match while dark regions show a high difference to the template.

Unprocessed color images and templates were converted into the hue saturation brightness (HSB) color space as a preprocessing step. Template matching was then performed on the brightness channel only. The authors applied the template matching method outlined by Bradski [11] as implemented in OpenCV (Open Source

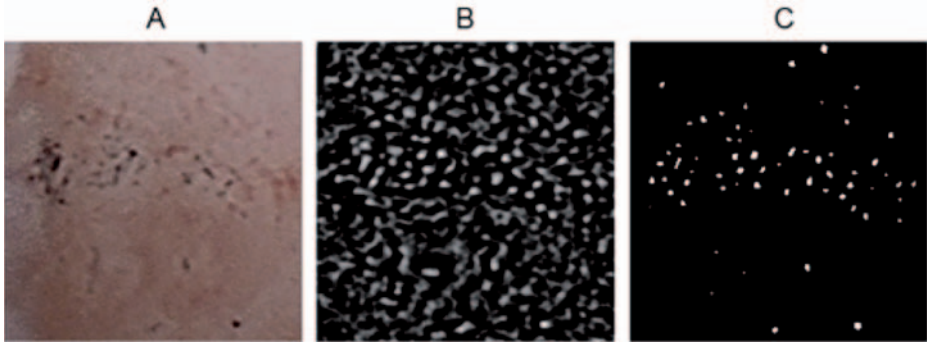

Figure 4. — The left image (A) shows the unprocessed sample with stained cells. The center image (B) is the NCC result as discussed above. Image C shows the outcome after applying the threshold to image B: White regions belong to stained cells, while black regions are not considered.

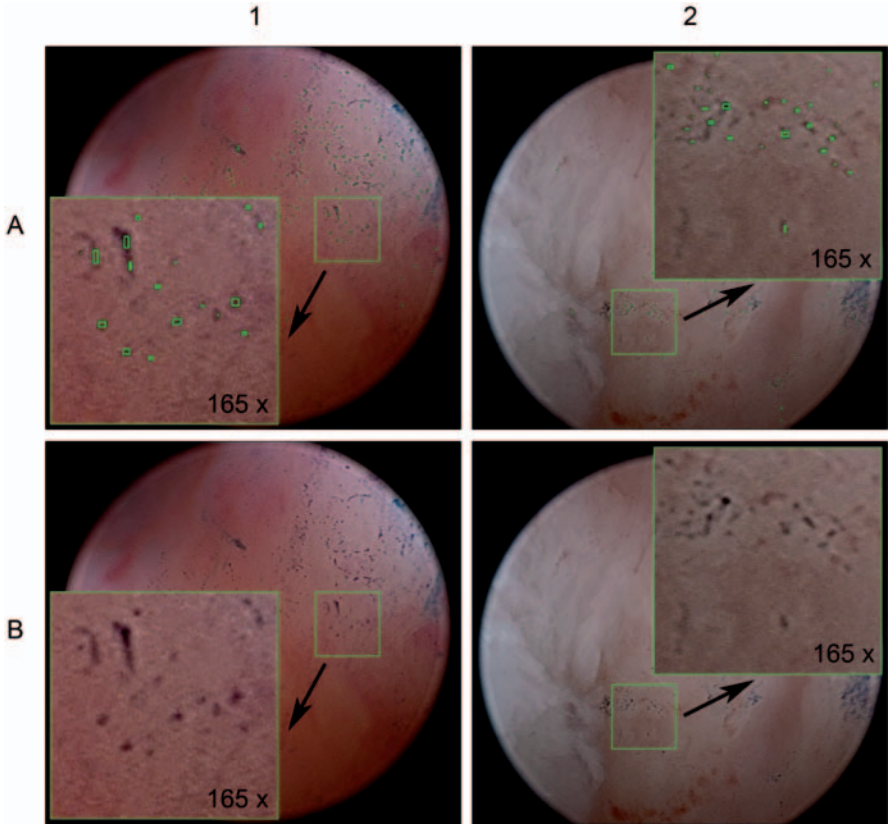

Figure 5. — In the second row (B) are two images with fallopian tube mucosa with different nuclear staining in 60-fold magnification. The top row (A) shows classified blue stained cells highlighted in green. For better visibility a selected part of the images is 165-fold magnified.

Computer Vision is a library of programming functions for real time computer vision) according to Rodgers and Nicewander [12].

In order to separate high from low similarity and to allow subsequent counting of similar regions, a threshold is applied to each pixel: the NCC result is converted to a binary image to facilitate the quantification of stained cells as shown in Figure 4. The image is segmented into two regions indicating either a match or no match with the template by applying

$$b(x,y) = \begin{cases} 1, & ncc(x,y) > th \\ 0, & ncc(x,y) \le th \end{cases}$$

where *th* is the predefined threshold (*0.68* in our case) and the result *b* denotes the binary result. Applying the threshold results in a binary image consisting of either *0* (no template match) or 1 (template match).

The authors use an empirically evaluated threshold (0.68) to indicate template matches for two reasons: First of all, the rate of false positive results was reduced when only high probabilities were considered as a match. False negative results were compensated by the use of multiple different templates. Secondly, a higher threshold results in fewer connected regions if the distance of two distinct matches is very small. This is because the probability of an actual match decreases from the center of the comparison region to its boundary.

As mentioned above, multiple templates were used for the identification of stained cell localization. To obtain the final result, all template matching results were merged by

$$r(x,y) = b_1(x,y) \cup b_2(x,y) \cup \mathsf{K} \cup b_n(x,y)$$

whereby $b_1 - b_n$ denote all *n* binary template matching results and *r* denotes the merged output. The union operator corresponds to the logical *OR* function, therefore same regions found by several templates were counted only once and regions found by one template only were considered as well.

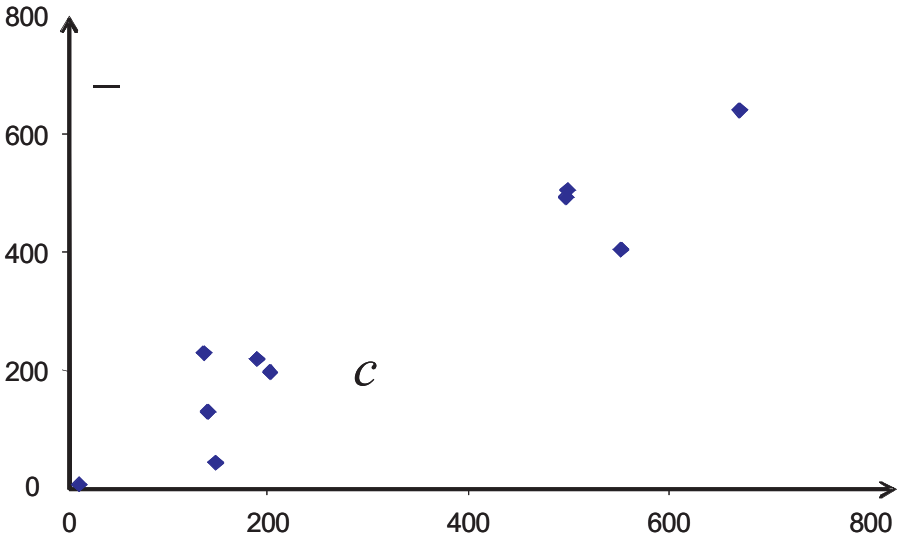

Figure 6. — Scatter Plot Diagram (Pearson Correlation $r = 0.96$) showing the relationship between the manual $\bar{m}$ and computerized $\bar{c}$ counts of the evaluated images of ten patients.

As the last step, all localized templates of the result image *r* were counted. In order to distinguish distinct regions, a border following the method as implemented in OpenCV after Suzuki *et al.* [13] was applied.

The number of each, of the two counts obtained by the medical expert staff examiner were denoted as m_1 and m_2 and the numbers obtained by the above described computer image processing method c_1 and c_2. For each patient the arithmetic mean $\bar{c} = \frac{c_1 + c_2}{2}$ resp. $\bar{m} = \frac{m_1 + m_2}{2}$ and the absolute value of the difference $d_c = |c_1 - c_2|$ resp. $d_m = |m_1 - m_2|$ for each of the two computerized and the two manual counts are calculated. A Pearson correlation coefficient between these mean values $\bar{c}$, $\bar{m}$ and a Wilcoxon signed-rank test for the obtained differences d_c, d_m was then performed.

Results

Two different characteristic examples of methylene blue stained fallopian tube mucosae are shown in the second row of Figure 5. The first row shows the blue dotted areas with green margins, allowing a numeric value to be attributed to tubal damage under magnified power = numeric amount factor (NAF).

The counts obtained by computerized analysis resulted in the same number for every image. However, the Wilcoxon signed-rank test for the absolute differences was highly significant ($p = 0.007$), and this can only be due to the variability in manual counting (Figure 6).

Discussion

Until now only a subjective grading of tubal damage exists with respect to nuclear staining. The present results suggest that computerized classification of methylene blue stained fallopian tube mucosal damage is a useful method to objectively evaluate and measure the extent of tubal destruction, as an additional evaluation during laparoscopy and fertiloscopy.

For image processing, a standard method was used (see above) for counting the methylene blue stained cells in the fallopian tube mucosa. The aim was to develop a simple, easy to learn, reproducible tool to evaluate inflammation, independent of the investigator´s experience.

The present study suggests that the computerized evaluation of nuclear staining of destroyed intrafallopian epithelial cells in female patients with primary infertility obtains similar, but more reproducible results, when compared with manual evaluation.

Admittedly, a possible disadvantage of automated pattern recognition is that it is limited by the quality of the images, because the classification system might not recognize blurred areas.

Concerning the methylene blue, the European Medicines Agency (EMA) has approved methylthioninium chloride Proveblue five mg/ml solution for injection for human intravenous therapy of methaemoglobinaemia. This new methylene blue shows increased purity and is especially free of heavy metals and organic impurities. It should be noted that to date methylthioninium chloride has been also used as a standard dye for chromopertubation in diagnostic gynecology. Fertiloscopy was performed by using two special single-use trocars.

Future studies might evaluate to what extent tubal damage could decrease pregnancy rates, and might facilitate the decision whether to perform in vitro fertilisation (IVF). Furthermore, studies should elucidate the relationship between multiple genital tract infections and especially the immunopathogenesis of the common chlamydia trachomatis infections in women and the development of infertility by the correlation of e.g. HLA determinants subtypes, human genetics, cytokine profiles, chlamydial heat shock protein

60, and infectious loads, as well as exact characterizing of in vivo physiological intratubal cellular changes [14-16].

Of course the present results obtained from images of ten patients only can be regarded as preliminary. However, even in this very small sample of patients the authors obtained a significant result. Since it is well known from power analysis that a significant result in a small sample is associated with a high effect size, this small sample might even valorise the present study.

In summary: cost effective computerized classification of methylene blue stained fallopian tube mucosal damage in combination with the simple operative fertiloscopic technique [17], or with laparoscopy, allowing additional procedures such as adhesiolysis and ovarian drilling in cases of polycystic ovarian syndrome (PCOS) might represent an improvement on HSG, and could be performed as a first line diagnostic and therapeutic tool before IVF procedures [18].

Conclusion

The computerized classification of methylene blue stained fallopian tube mucosal damage is an objective grading tool of tubal destruction and might prevent patients with unexplained or unclear infertility from unnecessary IVF in the future.

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