

Review

The Pap test: George N. Papanicolaou (1883–1962) A screening test for the prevention of cancer of uterine cervix

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1. Introduction

The death rate from cervical cancer has decreased significantly over the last 50 years as a result of widespread cervical cancer screening. Cervical squamous cell cancer is a classic example of neoplastic progression. The cervical epithelium produces a precursor lesion which, if left untreated, has the potential of progression to frank invasive carcinoma. Eradication of this precursor obviates the neoplastic progression. The precursor can be readily detected by a painless, reproducible and reasonably accurate screening test, the Pap smear, which has been the basis for the significant decrease in mortality related to cervical cancer [1].

2. George N. Papanicolaou

In 1941, George N. Papanicolaou, along with Herbert F. Traut, published his cornerstone paper on “The diagnostic value of vaginal smears in carcinoma of the uterus” in the *American Journal of Obstetrics and Gynecology* [2]. Since then, the ‘Pap’ smear, named as such in honor of Papanicolaou, has saved millions of women who would have otherwise discovered their cancer of the cervix uteri at a non-curable stage. Mortality rate from carcinoma of the cervix has, indeed, decreased from 14 per 100 000 women in 1940s to four per 100 000 in 1989 [3].

The story of Papanicolaou’s discovery was long, dating since 1928, complicated, and accomplished through indirect routes. But so was the life of the “greater medical researcher of our century without a Nobel prize”.

George Nicholas Papanicolaou was born in 1883 in Kymi, a small town of the Greek island of Euboea by the sea. His main interests were philosophy and the violin, but his father forced him to go to Medical School which he finished at the age of 21 with honors. But he was not

meant to practice medicine at home. His unsettled mind lead him to Germany to study the philosophy of biological science. He ended up with a Ph.D. in Zoology on “Sex differentiation of the *Daphnia*” (a water flea) in 1910 [4,5].

Later that year, he married his wife Andromache (Mache), who, since then, provided him with constant support and shared his private life and his scientific work. Not being able to find an academic position in Greece, they tried their fortune in France where Dr Papanicolaou worked as a physiologist on a Mediterranean oceanographic research ship for a year. But he returned to Greece once more to serve as a physician in the Balkan war. Having heard from American volunteers in the war about the opportunities that existed in the United States, he moved once more in 1913, this time for his last stop, the United States of America.

His first job there was selling carpets but he soon quit and worked as a researcher in a laboratory at the New York Hospital. His main area of research was with oocytes of guinea pigs. In order to use them, oocytes had to be collected at a certain stage of the cycle. A large number of guinea pigs had to be sacrificed because they had no external bleeding and a menstrual cycle could not be charted and the proper time for oocyte collection could not be estimated [5].

In 1916, Dr Papanicolaou thought of using a nasal speculum in the vaginas of guinea pigs in order to observe any slight bleeding. Indeed, every 15 or 16 days a scant bleeding occurred, so he could, then, approximate the proper time for egg collection. But he decided to take his finding one step further. He went on to examine these cells microscopically and found that cyclic ovarian changes could be revealed by studying the changes in the scraped cells. After a while, the hormonal status of the ovary could be predicted by observing the scraped cells and the eggs could be retrieved at the exact proper time [5].

In 1920 he started studying vaginal scrapings from

humans. When he first saw malignant cells in one of these smears, he realized he was in front of a major breakthrough. He started collecting data on cervical carcinoma cells and presented it for the first time at the 3rd “Race Betterment Conference” in Battle Creek, Michigan, in 1928. His colleagues, though, did not share his enthusiasm and Papanicolaou was discouraged and abandoned this subject for many years [6].

After having turned to hormonal changes again for some years. Dr Papanicolaou returned to the detection of cancer. After many years of observation, he established the correlation between scraped cells and the detection of carcinoma of the cervix uteri and published it in a classic paper in the *American Journal of Obstetrics and Gynecology* in 1941, entitled “The diagnostic value of vaginal smears in carcinoma of the uterus” [2] (Fig. 1).

In this paper he emphasizes the need to develop a “simple, inexpensive method of diagnosis that could be applied to large numbers of women in the cancer-bearing period of life”. He had realized the need for ‘early diagnosis’ for cancer and he applied that concept throughout all his work. He describes in detail the technique for collecting the cellular debris, smearing it upon glass slides and staining it in such a way that the various components may be studied. He also describes in great length the findings indicative of carcinoma of the cervix and those that are suggestive of carcinoma of the fundus and also of the vagina or vulva. He points out, though, that “the vaginal smear method is not as accurate for the diagnosis of carcinoma of the fundus as it is for that of carcinoma of the cervix”, something that we still believe after all these years [6]. This time his method was well accepted and training of cytopathologists in order to be able to interpret the cellular characteristics commenced.

In 1942, he published his monograph “Diagnosis of Uterine Cancer by the Vaginal Smear” which he himself considered as “his last contribution to science” [5].

His goal, thereafter, was to train other physicians in this

new technique and to make it available to the whole world. In 1962, he and his wife established the Papanicolaou Research Institute in the University of Miami, Florida, but, unfortunately, he died the same year from a myocardial infarction.

He received many awards and honors including the publication of two stamps, one in Greece in 1973 and one in the US in 1978 [5]. His figure is also on one side of the 10 000 Drachmae banknote.

3. Recent developments in screening for prevention of cancer of the cervix

Cervical cytology is still considered the principal method for early diagnosis of precursor lesions of carcinoma of the cervix uteri. As mentioned above, these lesions appear a considerable length of time before the manifestation of cancer of the cervix and, therefore, their prompt diagnosis and proper management can lead to an effective prevention of the disease.

The use of Pap smear as a screening test for cancer of the cervix in the last 50 years has led to a significant decrease in death rate from this disease, the decrease being as high as 70% in some countries. Indeed, the Papanicolaou smear, is probably the most successful cancer screening test used nowadays [7].

This test is cheap, simple to use, minimally invasive but, as is the case with all medical assays, its prognostic value is still under consideration [8]. It has to be noted, though, that the Pap smear is not a diagnostic but a screening test that reveals those women that need further assessment. If a low grade or high grade cervical intraepithelial neoplasia is found, for example, this does not exclude the existence of a preinvasive or even invasive carcinoma. Therefore, all women with an abnormal Pap smear, even a minimally abnormal one [9], should be followed up or further checked with other methods.

The main drawback with this test is the false-negative results. False negative are considered the results if, on double-checking, cellular elements indicative of cervical intraepithelial neoplasia or in situ adenocarcinoma or invasive cancer are found. About one-third of false-negative results of Pap smears are due to wrong interpretation of the cytological specimens, while the remaining two-thirds are due to improper collection and processing of the cellular material. Their rate of occurrence is between 5 and 30% [10].

It seems unlikely that the sensitivity of the method will be significantly increased by improving the microscopic examination of specimens by cytopathologists. The interest lies, nowadays, in finding ways to improve the collection, handling and processing of the specimens. The use of special tools for collection, such as the cytobrush, have, indeed, led to improved collection of the cellular material. With the advance of technology, new methods started

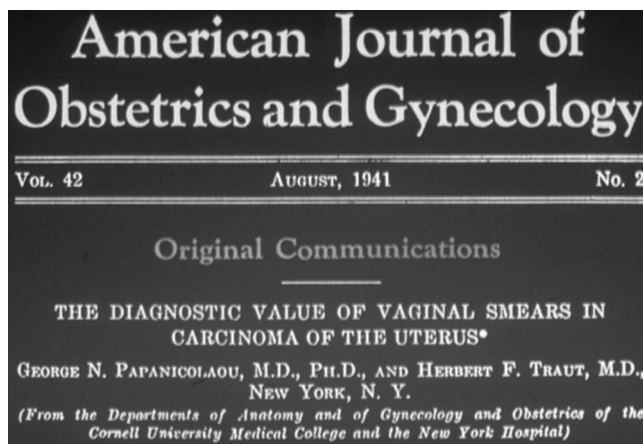


Fig. 1. The first page of Papanicolaou's cornerstone paper in the *American Journal of Obstetrics and Gynecology* in 1941.

being used for collecting and preserving the material needed.

Thin layer technology consists of collecting the material with a soft brush by performing a conventional Papanicolaou test and, then, rinsing it into a special fluid preservative form. From the latter, a thin-layer slide can be performed by using an automated slide processor [11–13]. This method provides a better quality of cell preservation and presentation, fewer inadequate samples, elimination of obscuring material and homogeneity of the sample [14]. An additional important advantage is the ability to use the remaining material for HPV DNA testing should the results of the test suggest that. Therefore, a single sample can be used for cytopathology and HPV testing [15].

Recently, computer-assisted automatic scanning devices, such as the PAPNET Testing System, have been employed mainly as a ‘quality control’ system. Their use for primary screening of cervical smears is not established yet but many trials are conducted so that automated primary screening of cervical smears is a possibility for the near future. The instruments used belong to a new class of medical devices designed to provide computer-aided diagnosis. The testing systems seem to have a high sensitivity and specificity and they could help decrease the false-negative smears [16–18].

Other methods and devices have also been employed in conjunction with the conventional Pap test in an effort to increase the sensitivity of primary screening or to extend its application to more groups of women. One of these is speculometry, a magnified chemiluminescent visual examination, which can be used to screen pregnant adolescents for abnormal cervical pathology [19]. Another is cervicography, or high-resolution cervical photography, the role of which as a screening device remains to be defined [20].

Finally, in the area of HPV diagnosis, new methodologies have been employed to complement the findings of the conventional Pap test. Intranuclear viral particles can be demonstrated by electron microscopy, immunohistochemistry and, recently, by molecular biology [21]. HPV DNA testing can be mainly achieved by the polymerase chain reaction (PCR) [22] or by the hybrid capture systems (HCS) [23]. The PCR method has a high sensitivity for detecting very low concentrations of HPV DNA. This, on the other hand, leads to a high number of false-positive results. The HCS method is a liquid-phase hybrid system for the detection of higher HPV DNA concentrations. This method, although not as sensitive as the PCR method, may be more helpful in identifying those women with concurrent squamous intraepithelial lesions (SILs) [24–26].

It is known that only a small percentage of women with HPV infection will present with a clinically important intraepithelial lesion. It is also established that cytology alone does not have a high enough sensitivity to diagnose all precancerous and cancerous lesions. It is, nowadays, believed that the diagnostic accuracy for cervical cancer and its precursors is improved by using a primary diagnos-



Fig. 2. George Papanicolaou along with Professor Nicolaos Louros in the latter's office in ALEXANDRA Maternity Hospital of the 1st Department of Obstetrics and Gynecology of Athens University Medical School during his visit in Greece in 1957.

tic test (conventional Pap test, liquid-based Pap test, automatic scanning devices) along with an HPV assay as an adjunctive test, especially in the cases with equivocal cytological cervical atypias [27].

But even after the introduction of new technology and the increase in sensitivity of the screening tests with new methodologies, the fact is that thousands of women have never received a Pap smear or receive it sporadically. To reduce deaths from cervical cancer, lowering the false-negative rate may not be as important as having regular Pap smears [10]. Papanicolaou's discovery of the screening test is now recognized by many as the most significant advance in the control of cancer in the 20th century [28]. Every time a woman dies of cancer of the cervix, we must turn our minds to the millions of others that have been saved due to a man's brilliance, determination to his scientific work and devotion to mankind. And we must then think of George N. Papanicolaou with gratefulness (Fig. 2).

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