DIAGNOSIS AND TREATMENT OF OVARIAN NEOPLASTIC ALTERATIONS

Editor:
H. DE WATTEVILLE

Diagnosis and treatment of ovarian neoplastic alterations

Proceedings of the American-European Conference on the Ovary, Montreux, Switzerland, December 9-13, 1974

Editor:
H. DE WATTEVILLE, *Geneva*

Co-editor:
P.R.J. BURCH, *Leeds*

1975

Excerpta Medica, Amsterdam — Oxford
American Elsevier Publishing Co. Inc., New York

International Congress Series No. 364

ISBN Excerpta Medica 90 219 0296 6
ISBN American Elsevier 0 444 15194 X
World Registry Number B 744228

Publisher:
Excerpta Medica
305 Keizersgracht
Amsterdam
P.O. Box 1126

Sole Distributors for the USA and Canada:
American Elsevier Publishing Company, Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

Printed in The Netherlands by Krips Repro bv, Meppel.

The Symposium was sponsored by the Foundation International Cooperation in the Medical Sciences, Montreux, Switzerland.

Contents

III. Ovarian neoplasia, therapeutic problems

IV. Germ cell tumors and tumors with endocrine secretion

Introductory remarks

H. DE WATTEVILLE

Clinique de Gynécologie et d'Obstétrique, Hôpital Cantonal, Geneva, Switzerland

Cancer of the ovary is a frequent tumour ranking third in women after breast and uterine cancer. Treatment is still not very satisfactory. Five-year survival is rather low (less than 20% in most series), despite the introduction of chemotherapy and improved methods of irradiation. One of the main reasons for this situation is the fact that the disease is very often only diagnosed at an advanced stage. Little is known so far concerning the epidemiology and it is therefore difficult to determine high risk groups within the population for which special detection campaigns could be organized. Although ovarian cancer becomes more frequent with advancing age, it may develop in very young women. There are many different histological aspects of ovarian cancer and the diagnosis of malignancy is not always an easy task. Furthermore, there is great variability between the different types of ovarian cancer in the clinical evolution and reaction to irradiation and chemotherapy. Thus it is difficult to devise uniform schedules for treatment.

Let me now comment briefly on the programme of the meeting. A good knowledge of embryology, anatomy, histology, and endocrine function of the ovary is a prerequisite for gaining insight into the pathways by which an ovarian malignancy may develop, and also for a better understanding of the functional and morphological changes which this development will induce. Accordingly, the first lectures and the first discussion will be devoted to this subject.

For several histological types of ovarian tumour the assessment of malignancy may be difficult, and for some forms of tumour discrepancies arise between the histological aspect and the clinical evolution. It seems appropriate, therefore, to review briefly the non-malignant ovarian growths, the more so as these tumours are often associated with an ovarian cancer.

We shall also hear about attempts to produce experimental ovarian cancer: such studies may shed light on the pathogenesis of this malignancy. We shall hear a description of the various cell types from which the different classes of ovarian neoplasms originate, and a report on the differential behaviour of these individual cell types. These lectures may provide a sound basis for a unanimously acceptable and practical histological classification.

Ovarian growths with an endocrine function lead to specific clinical syndromes. Their degree of malignancy is variable, and often difficult to predict. Theoretically, at least, some of them might be stimulated by pituitary hormones. It seems wise to devote some time to the discussion of problems relating to these functioning tumours,

with special emphasis on controversial subjects such as the minimum necessary extension of surgery and the place of radiotherapy.

I have already mentioned that the rather unsatisfactory therapeutic results obtained so far are to a great extent due to late discovery of the disease. Thus it appears of paramount importance to review carefully all diagnostic procedures used at present and their respective value, whether it be the simple clinical examination, an exploratory laparotomy, or the use of X-rays, ultrasound, cytology, or laparoscopy. We await really reliable biochemical or immunological tests now that investigations in these directions are under way. Early detection of a recurrence, or persistence of the tumour after treatment by a 'second look' procedure should be discussed, together with the diagnostic methods.

Last, but not least, our attention must focus on the treatment of ovarian cancer. Everyone agrees that surgery still comes first in our fight for the eradication of an ovarian neoplasm. However, opinions differ widely on the best time for the operation and how radical it should be.

1. Should the omentum be systematically excised?
2. Is it justified in certain cases of early unilateral cancer in young women to remove only the involved ovary?
3. Does it make sense to perform a hemipelvectomy, or pelvectomy, if the ovarian growth has invaded the bladder and/or the rectosigmoid?
4. Is it preferable to attempt in far advanced cases the removal of as much cancer tissue as possible, or is it better to perform only a biopsy and to try radio- or chemotherapy?
5. Is it useful to start in clinically far advanced cases with chemotherapy and/or irradiation, possibly followed later by a surgical attempt to remove the residual tumour?

A discussion of these and similar questions will give us an opportunity to hear about the experience gained by congress participants with one or the other of these procedures: such knowledge may be helpful for the handling of future cases. Radiotherapy has, beyond any doubt, proved its value, either as an addition to surgery or even alone in advanced cases, but again many problems concerning the optimal schedule remain open for discussion. The same holds true for chemotherapy and the combined use of both forms of treatment. Furthermore, it will be interesting to hear about the possibility of testing, in a tissue culture of an individual ovarian cancer, its reaction to various cytostatic agents. This procedure should enable us to select the most efficacious drug for a given case.

Finally, we should try to assess to what extent progestagens are efficacious in the treatment of an endometrioid ovarian cancer. Taking into consideration the multiple problems mentioned, it is fortunate that experts in the field of ovarian cancer, coming from the United States and several European countries, will meet for 3 days to present and discuss their data. The American-European Conference on Ovarian Cancer will provide an opportunity to review all the aspects of the fight against ovarian cancer. The lectures should spread present knowledge of this disease and the best diagnostic and therapeutic procedures among a great number of gynaecologists. Furthermore, the exchange of ideas among the experts participating in the meeting may lead to new projects for basic and clinical research dealing with ovarian cancer. In conclusion, we hope that the Conference will contribute to a fruitful cooperation between various oncological and gynaecological departments and establish valid statistics and new and still better methods for the diagnosis and treatment of ovarian cancer.

I. Embryology - histology - endocrine function of the ovary

What is the ovary?

J. DONALD WOODRUFF

Department of Pathology, The Johns Hopkins Hospital, Baltimore, Md., U.S.A.

It is only fitting and proper that any discussion of the ovary should begin with its birth, its infancy and its growing pains.

There is little doubt in most physician's minds but that this relatively tiny organ, rarely in its adult state weighing more than 7-8 g and occupying a space of more than 12-15 cm^3, carries more explosive clinicopathologic impact than one of the most magnificent atomic bombs. Nor is there any doubt in the minds of both the clinician and the pathologist that it behaves in most bizarre and unpredictable manners.

The discussion will concentrate largely on the embryology of the ovary since I believe that knowledge of the embryology is mandatory to the understanding of the vagaries of functional and pathologic activities of this unique organ. I must state initially that I am not an embryologist nor a geneticist.

It is generally accepted by most investigators that the chromosomal complement of the individual determines the sex of the embryonic gonad, i.e. whether it will become an ovary or a testis. Thus, the XX complement or the XY complement is of basic importance in the determination as to whether the gonad will differentiate into an ovary in which the cortex predominates, or into a testis, in which the medullary portion is dominant.

The inductors, influenced by these chromosomes, are, as far as I know, not well documented. Whether these agents are steroids, proteins, or enzymes, or a combination, which I personally suspect, is as yet undetermined. We are aware of the fact that steroids have an influence on the external genitalia, and the latter can be described in children whose mothers received certain progestational agents during early pregnancy, or whether it happens to occur in later life, as described with specific functioning tumors. Nevertheless we also know that steroids, as such, have little to do with the variation in development of the internal genitalia.

Yost's excellent studies have demonstrated that the implantation of a testosterone pellet at the site of the developing gonad will not inhibit the Müllerian (paramesonephric) system. Conversely, the implantation of the testis will result in the development of abnormalities in the internal genitalia. Thus we must assume that it is not the hormone but another agent, possibly a polypeptide, which produces such alterations. Variations may occur including such dramatic changes as those seen in the individual with a well developed male system on one side while on the contralateral side, due to a variation in development of the gonad or some early degenerative process, the Mülle-

rian system develops. Thus we can understand how mixed gonadal dysgenesis may result in a so-called 'inguinal uterus' in the genetic male. Nevertheless, this special 'determining agent' undoubtedly has little or nothing to do with the development of or alterations in the primitive gonad, but relates to the inductors which alter phenotype milieu of the individual.

So with these few background words, I would like to demonstrate some of my opinions relative to the embryonic gonad, its development, the individual histologic alterations which take place during the normal cycle and those associated with the common neoplastic alterations. Adjacent to the gonad are the mesonephric structures, namely the glomeruli, the duct and its tubules. At this point, the embryonic gonad is totally undifferentiated, and there are no specific features which identify it as an ovary or testis. The overlying mesothelium, i.e. germinal or peritoneal epithelium, is of mesodermal origin, as are the underlying stromal cells. Consequently, the appearance of two elements in the common ovarian neoplasm is routine since both the 'stroma and epithelium' are of similar origin.

Von Wagenen's studies determined that the germ cell appears for the first time in the gonadal ridge at approximately 4 weeks post ovulation. The germ cell is of major importance since it is the stimulus which induces first the development of the gonad and later its differentiation into either a male or a female organ. Later the germ cell causes the proliferation of the stromal element and thus, without the germ cell, the gonad will be either agenetic or dysgenetic, the latter being the more commonly used term for maldevelopment.

The germ cell is first seen in the entoderm of the hind gut, as noted by Witschi, and thus many have felt that it was of entodermal origin. Nevertheless most investigators now suggest that this unique and ubiquitous cell arises in the wall of the yolk sac and migrates into the base of the mesentery where it is first appreciated at approximately 5-6 weeks of embryonic life. The basic reason for the migration of this cell specifically into the gonadal ridge is somewhat in doubt. Chemotaxis has been suggested as the motivating process for such action. However, this term has been employed to explain most migratory actions for which there is no definable explanation. The germ cell does migrate to other positions, but hopefully in the latter areas the cell dies. Nevertheless it may remain at the base of the mesentery, thus accounting for the development of germ cell tumors at these extragenital sites.

The number of germ cells in the primitive gonad has fascinated many students. It was Baker in 1963 who demonstrated that there were approximately half a million germ cells in the gonad at 2 months of embryonic life. This number rapidly rises to almost 7 million at about 6 to 7 months of embryonic life; however, attrition reduces that number to 2 million at birth. Attrition continues, as is well known, so that at 3 years of postembryonic life there are 700 to 800,000; at the menarche approximately 300 to 400,000; and at the time of the menopause the germ cells have, to all intents and purposes, disappeared. There are those who suggest that the absence of germ cells is not the cause for the menopause, but rather there are more important vascular changes to explain this physiologic alteration. I personally doubt the latter, since after studying the ovary for some 35 years I appreciate the routine absence of the germ cells in postmenopausal ovaries while the vascular changes are imperceptible.

Exactly why this attrition continues is unknown since, technically speaking, it is felt that 25-30 primordial follicles begin to mature with each cycle and that one or possibly

two proceed to the point of ovulation, while the remainder become atretic. Assuming 13 normal cycles each year and 30 germ cells either maturing or dying per cycle, approximately 400 germ cells are consumed per year. Thus over a period of 40 years, approximately 16,000 germ cells would be eliminated. If 300,000 cells were present initially approximately 285,000 should remain after 40 years of menstrual existence. Possibly the processes involved in the maturation and degeneration can be discussed later.

It can be appreciated easily that in the primitive ovary there is no so-called 'tunica albuginea or capsule'. The germinal epithelium cannot be differentiated from the underlying tissue, and thus there seems to be a gradual transition from the overlying mesoderm or mesothelium to the underlying stromal elements.

On the other hand, in the male gonad, the capsule or tunica albuginea is well developed by approximately 10-12 weeks of embryonic life. Thus there is a basic difference between the male and the female gonad, i.e. the predominance of the cortex in the female, in contrast to the preeminence of the medulla in the male. Furthermore we have been unable to appreciate any primitive sex cords in the female gonad although in the hilar region and adjacent in the para-ovarian (the broad ligament) region of the adult female are the mesonephric glomeruli, and duct and its tubules.

Basically it is distressing to appreciate that there are no sex cords in the female gonad. They were most convenient structures by which one could explain the development of certain unusual ovarian tumors. Dysontogenesis suggests that is due to a 'male directness' to all embryonic gonads, male or female, and if the basic sex cords in this gonad were segregated at this embryonic stage they could serve as the *nidus* from which such tumors as the arrhenoblastoma with its 'sex cords' might result. During the next stage of differentiation resulting in the development of granulosa cells, foci of the latter, residing in or near the hilum, might be stimulated in later life and a granulosa cell tumor would result. Lastly foci of primitive unencapsulated germ cells, continuing to proliferate their embryonic state, could result in the development of a dysgerminoma. It was a simple theory, and I regret that this theory of dysontogenesis is incorrect; however, Cohnheim's hypothesis which proposed that all tumors arose from 'embryonic remains' is unrealistic in today's society. Thus I find no justification for the use of the term sex cords in the female gonad at this early developmental stage unless they relate to the mesonephric structures in the para-ovarian region.

The latter are seen in the hilum of every ovary and in the embryonic gonad both the low epithelium of the tubules and the tall pale epithelium of the duct as it enters the mesonephric glomerulus can be recognized. This tall pale cell is rarely seen in the adult ovary, but of course the mesonephric tubules persist in approximately 100% of the cases. These should not be confused with the sex cords that have previously been described erroneously in the embryonic ovary. In the embryonic ovary, the most prominent feature is the germ cell, originally unencapsulated but later surrounded by the invading mesothelium to form the primordial follicles. When unencapsulated, the germ cells continue to mitose, and of course, if they are not encapsulated or do not die, the gonad will be overrun with germ cells, thus the origin of a dysgerminoma or probably more correctly a germinoma.

The stage at which the germ cell is encapsulated is a point of some controversy. On asking one embryologist the question 'When is the germ cell first encapsulated?' one may receive a variety of answers. There are those who have suggested that this occurs

at approximately 20 weeks post ovulation. I believe this is erroneous, since our studies have demonstrated encapsulated germ cells (the primordial follicles) in the embryonic gonad of between 7 and 8 weeks.

In the young gonad, all of the elements that later may be involved in the development of ovarian tumors can be identified. The germ cell is, as noted above, the cell of origin of the germinoma (dysgerminoma) and of most, if not all, of the teratomas. The dysgenetic gonad and the gonadoblastoma (the latter is realistically not a true tumor but rather a variant on the dysgenetic gonad) are fertile fields for the development of germ cell tumors. Secondly are the mesothelial and stromal cells which can be appreciated in the ovary. There are basically 2 varieties of stromal cell, one is the supporting connective tissue found in all organs, and in the ovary undoubtedly arises from the medulla, while the other and more important is the stromal or potentially functioning element of the gonadal matrix. I feel that the latter differentiates from the overlying mesoderm (mesothelium) and to understand this basic thesis is to understand that the two elements in almost all gonadal tumors are of the same embryonic origin. Thus most of the common ovarian tumors have 2 basic cellular components.

In the gonad at 14-15 weeks of embryonic life, the overlying mesothelium, the underlying gonadal stroma, and the many encapsulated and unencapsulated germ cells (those which continue to proliferate) can be easily recognized.

It is also appreciated that the ducts, both the Wolffian (mesonephric ducts) and the Müllerian (paramesonephric ducts), arise within the 'same capsule'. This proximity explains the frequency with which abnormalities develop in both systems; thus if an anomaly is noted in the female genital canal, such as a unicornate uterus, the urinary tract should be studied promptly since in many instances an anomaly of the urinary tract also will be found. If the ureteric bud (from the mesonephric duct) does develop, and rise to the metanephric mass (the kidney), the latter either will not develop, or, with a shortened ureter, the kidney will be displaced into the pelvis rather than occupying its normal position.

After birth the many primordial follicles and the 'so-called germinal epithelium' can be easily recognized but more important is the absence of any definite capsule. In the primitive gonadal stroma the difference between the theca interna and the theca externa seems indefinite except for the geography of the area. However, it is well known that the theca interna does respond to hormonal stimulation, primarily the luteinizing hormone with resultant 'luteinization or yellowization', and that, under certain circumstances, the theca externa may respond in exactly the same way. The reason for the rather remarkable response in the 'stroma' adjacent to the developing follicle may be related to the local enzymatic milieu rather than to the systemic agents, e.g. the hormones. Personally I believe the nerve supply to the basic stromal cell has something to do with its ability to react, and this theory has been strengthened by electron microscopic studies of the ovary.

The adult functioning ovary contains a variety of structures: the primordial follicle and the developing follicle, in addition to the corpora fibrosa and albicantia. The capsule is poorly defined and is not a homologue of the tunica albuginea in the male. Basically the capsule is a layer of condensed fibrous tissue which varies depending on the patho-physiologic situation. In this adult ovary, in addition, there are those germ cells seemingly 'caught in the fibrous capsule'. It would not seem likely that a primordial follicle encapsulated by fibrous tissue would develop adequately; certainly it

would seem almost impossible for the surrounding 'theca' to react to the gonadotrophins.

Finally it should be appreciated that the polyovular follicle or 'two eggs in one basket' is not an uncommon finding in the young adult ovary. Although rarely seen in the normal female gonad after 30 years of age, they are found in 8-10% of all younger ovaries, and may play a role in the genesis of teratomas.

Progressing from this primitive gonad to the earliest changes of maturation, the well developed follicle in the antrum formation consists of the cumulus or discus proligerus, the corona radiata and the granulosa with its 'Call-Exner' bodies. After rupture of the follicle, the earliest corpus luteum is characterized by a basal layer of granulosa cells that appear a little taller than the unluteinized upper layers, stimulating a 'basal type of epithelium'.

Follicle maturation prior to ovulation, i.e. approximately the 14th day of the stylized 28-day cycle, is characterized by mitoses in both the granulosa and the luteinized thecal cell. Following ovulation, luteinization of the granulosa is the first stage of corpus luteum formation, and is demonstrated by an increase in cytoplasm, first in the lowest layer of the granulosa. Vascularization of the corpus luteum occurs on the 17th and 18th day of the cycle (the corpus hemorrhagicum) followed by the laying down of a layer of fibroblasts lining the central cavity. The first degenerative change that can be appreciated occurs on the 23rd or 24th day of the cycle and is characterized by the formation of the so-called 'mulberry cell' produced by the vacuolization in the luteinized granulosa cell. Mitoses are absent in the granulosa after the 18th day; however, they may be seen in the active theca throughout the cycle.

The normal ovary of the woman over the age of 30-35 years is characterized by the usual functioning elements and germinal inclusions. Goodall, more than 50 years ago, made the observation that such inclusions were routine findings in the ovary and also that they might be lined by tubal and mucinous epithelia. These elements are of major importance since they represent potential sites of tumor formation and the epithelia in the inclusions are classic of those seen in the common 'epithelial' tumors of the ovary. Evidences of function in the ovary may be appreciated by a variety of histologic alterations, most commonly in the stroma. Such structures as the cortical granuloma, characterized by an accumulation of lymphocytes surrounded by proliferative theca, are felt by some to be associated with functional activity.

In the 'normal' postmenopausal ovary, many evidences of past functional activity are recognized particularly in the presence of corpora albicantia and corpora fibrosa. The vessels, although thick walled, are rarely occluded. There is little histologic evidence of hormone production. Nevertheless, on occasions, the abundant stroma (classified occasionally as stromal hyperplasia) may be associated with endometrial hyperplasia, suggesting estrogen production. Consequently the normal postmenopausal ovary, although devoid of germ cells, the commonly considered stimulant of gonadal stromal activity, is undoubtedly producing steroids even though at a reduced level.

In the maturing follicle of the normally functioning ovary of the adult menstruating woman, the unluteinized granulosa of the antrum is surrounded by the large luteinized thecal cells which have abundant eosinophilic cytoplasm, a light blue nucleus and a brilliant nucleolus indicative of a metabolically active cell.

A histologically similar cell may be seen around the nerve in the hilum of the ovary

and this element is a variant on the gonadal stroma. However, although histologically similar to the luteinized theca, it apparently is functionally different. The same cell may be appreciated in the gonad of the postmenopausal patient who demonstrates functional activity by a shift to the right in the vaginal maturation index, the presence of hyperplasia in the endometrium, and elevated urinary estrogen levels. Assuming that the male gonad is a medullary structure, these 'hilar cells' are male oriented as demonstrated by the presence of Reinke crystalloids. The luteinized cells, in such cases of thecosis, often seem to be organized around dilated lymphatics, and simulate the process seen around the developing follicle. Undoubtedly the study of such foci of functioning elements will reveal the presence of 'nerves' in the adjacent area. Consequently, a 'neurohormonal' mechanism may be the activating process to stimulate such cells to functional activity.

In the Stein ovary with its plethora of stroma, luteinization is appreciated around many of the follicles, as well as in the adjacent stroma well beyond the functioning elements, demonstrating that this 'theca externa' has the potentiality, in certain circumstances, of 'luteinization'. In a variety of diverse circumstances the ovarian stroma cell appears to be histologically and clinically functioning. For example, in the 'mucinous cyst' associated with pregnancy, the stroma may appear 'luteinized' and although the cells simulate those seen in the theca interna, they seem to exert a masculinizing effect on the host. Similar clinico-pathologic findings may be appreciated in the pregnant patient with a Krukenberg tumor. In an attempt to demonstrate the differential activity of the cells in such tumors, enzyme histochemistry has been employed. Although in such studies definite hormonal activity is not demonstrated, nevertheless there are correlative data between the reactions in the functioning cells of the normal ovary and those of the ovarian tumor. Again these correlative data are helpful, in demonstrating that there is a differential activity in the stroma, and that this may account for some alteration in the host.