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From the new moon to the full moon: Anatomic Pathology in Bologna from the 1500s to Armando Businco

P. Scarani* **, V. Eusebi

* Dipartimento di Ematologia e Scienze Oncologiche L. e A. Seragnoli, Sezione di Anatomia, Istologia e Citologia “Marcello Malpighi”; ** Museo delle Cere “Luigi Cattaneo”, Università di Bologna, Italia

On July 12, 1989, Antonmaria Mancini (1929–2007) celebrated the 130th anniversary of the Department of Anatomic Pathology of the University of Bologna. July 12, 1859 was also notable as the day that Bologna was formally considered as part of the kingdom of Vittorio Emanuele II. On that day, in those confused times, Cesare Taruffi (1821–1902) received orders from Luigi Carlo Farini to establish the Department of Anatomic Pathology. During the festivities, the University of Bologna also inaugurated the Museum of Anatomic Pathology. For the occasion, Prof. Mancini received many compliments from a veterinarian on the presentation of the museum. The Professor answered by pointing towards a lesser colleague saying, “I am not the one to thank: I told him to do it, and he acted in full autonomy”. The veterinarian responded, “It is certainly a great merit on your part to have given him such liberty”. Antonmaria Mancini was like this, often saying, “If God gave free men the capacity to do evil, then I must do the same with my collaborators”.

Prologue

Without achieving practical results for the sick, medicine would not have a great reputation, and indeed such a situation was evident until the end of World War II. The discoveries of Koch in the late 1800s, for example, were not well received due to the lack of knowledge in pharmacology, and despite advances in knowledge of disease, the available therapies still had an efficacy that was only slightly better than that of faith healers or charlatans. After the war, an epoch of optimism emerged following a series of tangible therapeutic successes. It is this long period of darkness that inspired the somewhat unconventional title of this article on lunar phases. This is not related to the quality of previous discoveries, but rather to their practical applicability and the difficulty in defining anatomic pathology, and starting from the 1800s, how to define anatomic pathologists.

The new moon: morphology in the 1500s at Bologna

The study of forensic medicine at Bologna was reported as early as the time of Dante (1265–1321) when physicians were called upon to determine if the sudden and unexpected death of some famous person was due to either natural causes or poisoning. It is not surprising that these experiences have been remembered, as they are connected to legal services, the principal science of the antique University of Bologna; medicine was considered as just a minor art. Nonetheless, apart from such activities, what physicians actually did was not of great interest.

Until the time of Giulio Cesare Aranzi when anatomic studies began to flourish, in spite of the contribution of Mondino de’ Liuzzi (1275–1326), there had been no correlation between pathological and anatomical studies. The first autopsy to understand the cause of death was performed by the Florentine Antonio Benivieni in Bologna (1430–1502).

Giulio Cesare Aranzi (1530–1589)

Aranzi (Aranzio is the Latin name) was an authentic Bolognese (Fig. 1). He splendidly described foetal circulation, although the only trace of his studies that has persisted is the venous duct of Arantius. He also discovered the hippocampus, which in lay terms, was referred to as the ‘silkworm’, and it is curious that this analogy was easy to understand in Bologna given the large development of the silk industry there at that time. At any rate, the discovery was a revolution for neuroscience. By identifying the hippocampus, Aranzi had become aware of the existence of another horn (the temporal horn) of the lateral ventricle, which was also known by his mentor Vesalio. This was an enormous discovery considering the difficulties encountered in macroscopic study of the brain before the development of fixatives.
in the 1800s. The vague knowledge of brain anatomy is also apparent from Vesalio, who illustrated the nervous system in “De humani corporis fabrica” in 1543. In this regard, it is still difficult for us to understand how the representation of a human brain in the group of subjects surrounding God in the creation of Adam in the Sistine Chapel could have been attributed to Michelangelo in 1512, especially considering the comparison was made using drawings made in the 1900s by Netter and not those made during Michelangelo’s time. At any rate, in addition to the individuation of anatomic structures, Aranzi published the first anatomic-pathologic work from Bologna that combined anatomy with a description of pathologic processes.

Another work by Aranzi, edited in Venice the same year, together with anatomical studies, *De tumoribus secundum locos affectos*, consisted in a heterogeneous collection (hydrocephalies, cysts, abscesses, solid masses) of tumefactions (tumours) divided by anatomic area. These studies were the result of the activity of a practical physician who was also dedicated to surgery. Today, almost no one remembers Aranzi at Bologna.

In the Bolognese medicine of the 1500s, there was truly a darkness associated with the new moon if one considers the dominant figurehead of the university, Gerolamo Cardano. Students, however, preferred Ulisse Aldrovandi (1522-1605), although he was a naturalist more than a physician. Nonetheless, he had a modern vision of morphology and carefully studied the internal organs of animals. His teratology studies were extremely rigorous. Even Aldrovandi did not have significant success at Bologna, in spite of his international reputation, and was only able to publish a small fraction of his work. Likewise, he did not want any help from his Bolognese cousin, Pope Gregory XIII (the same who reformed the calendar), who had huge practical advantages from the scientific studies of Aldrovandi. The precursor of Bolognese studies on anatomic pathology, Marcello Malpighi, should also be remembered as curator of the naturalistic collections of Aldrovandi.

**The crescent moon**

*Marcello Malpighi* (1628-1694) and his followers. Malpighi, born in Crevalcore (Bologna), was a great anatomist, anatomic pathologist and clinician (Fig. 2). He was the first to use a microscope for the study of internal organs, and his name is still associated with structures of the kidney, spleen and skin. He is also known for his studies on the lung and embryonic development. Malpighi, however, should be considered as an anatomic pathologist that carried out accurate autopsies accompanied by clinical data. His referrals were often preceded by both biographical information and detailed clinical history, and his autopsy methods were similar to those used today. He proceeded by observation of the cadaver before examining the internal organs starting with the intrathoracic ones. He was primarily a practical clinician, and all of his interests in normal anatomy and pathology derived from problems that had clinical relevance. Unfortunately, his work was ahead of his time. Despite his undisputed successes and the fact that his name is still found in modern texts on anatomy and embryology, it was one of his students, Giovanbattista Morgagni, who brought under question his microscopic findings thereby postponing such discoveries until the second half of the 1800s.
Malpighi’s talent as an anatomic pathologist was repeatedly demonstrated. One such example can be found in a study by Salfi\(^\text{13}\) on a brilliant diagnosis by Malpighi on Paget’s disease of the bone, which was certainly ahead of his time, and often misunderstood by today’s paleopathologists. There are still many unexplored drawings and manuscripts by Malpighi in the archives at Bologna.

Anton Maria Valsalva (1666-1723) was the greatest pupil of Malpighi. He was born in Imola (Bologna), and like his master lived on the margins of academic life. In 2004, a memorial edition of his work on the human ear was published (not in Bologna) which was certainly a monument to his meticulous study of morphology, comparable to that of Aranzi’s studies of foetal circulation. The favoured apprentice Giovanbattista Morgagni described the death of Valsalva with extraordinary affection, noting his passion for morphology. Valsalva carried out his autopsies in a ‘total’ immersion and even tasted what he saw. In spite of the fears of modern pathologists, he did not die of infection.

Giovanbattista Morgagni (1682-1771), from Forlì, can be considered as the deity of autoptic anatomic pathology of the Bolognese school. Bologna nonetheless rejected him and never treated him well, despite his affection for the city, thanks to his bond with Valsalva.

**Towards the full moon: from Napoleon to the Roman Republic**

In 1888, when the 800th anniversary of our university was celebrated, the department of anatomical pathology in Bologna was just a few years old, as it was founded in 1859 when the university was reorganized following the cessation of its administration by the Vatican\(^\text{112}\). Unfortunately for Bologna, this was not the first department of anatomical pathology established in Italy, and was preceded by Florence, where it was instituted by Granduca Leopoldo II in 1840, and by Genoa\(^\text{5,15}\). In Bologna, anatomic pathology was founded at the same time as the University of Modena\(^\text{16}\). However, anatomic pathology in Bologna, independently of when it was formally founded, had already been firmly established since the beginning of the century. Along these lines, a University Museum of Pathology was founded by Napoleon (then president of the newly-formed Roman Republic) in 1804\(^\text{17}\) in which dried samples and wax models from organs affected by various diseases were collected. Other than the materials still conserved at the Museum of Anatomy, little remains of the museum founded by Napoleon, with the exception of a series of notes in Latin in which the collection of samples is described. The notes were published in the *Opuscoli Scientifici dell’accademia delle scienze dell’istituto di Bologna* between 1818 and 1823\(^\text{18,21}\), and were written by Luigi Rodati who was the curator of the museum from 1815 to 1832. The dissertations of Rodati, however, were limited to dried samples. During that time, the museum was already one of the largest in Europe and housed wax models that are still in excellent condition, the most well renowned of which is a bust of a case of acromegaly (1811).

The use of anatomical wax models was flourishing at that time, and as in Florence with the birth of anatomic pathology, many types of pathologies began to be modelled\(^\text{22}\). Wax models from the early-mid 1800s can still be found in the museum (presently located in the Institute of Anatomy known as the Luigi Cattaneo Anatomical Wax Museum). They were made by Giuseppe Astorri, who was part of the anatomical wax school in Bologna dating to the 1700s. The activity of the Bologna school has been documented in the form of a catalogue conserved in the State Archives in Bologna. After the fall of Napoleon, the Bologna institutions were placed under the control of Cardinal Carlo Oppizzoni, a man of outstanding moral character. In this regard, he sharply criticized, and from a theological standpoint motivated, the excommunication led by Pope Pio IX against the Roman Republic\(^\text{23}\).

Oppizzoni passionately cured the growth of the medical faculty in Bologna, providing it with guidelines that were also considered a model for more recent Chairs of Medicine. As one example, he proposed that an award should be given to the physician that performed the most autopsies during the year. Antonio Alessandri (1786-1861)\(^\text{24}\), a legendary figure of the medical school in Bologna, was particularly outstanding in this regard. In contrast to the majority of professors at the university, he was from very modest origins and is best remembered as the founder of the museum of anatomic veterinary pathology (which he called comparative pathology) and palaeontology. Luigi Calori, author of a splendid biography on Alessandri, is probably the only morphologist that can be compared with him\(^\text{25-27}\). A systematic study of the immense activity of Antonio Alessandri has never been carried out. Nonetheless, the large amount of work that he left behind is amazing, and for example many skeletons of exotic animals, still housed in the Museum of Zoology and Comparative Anatomy, appear to correspond to the multitude of his original publications. Alessandri was part of the generation that went from macroscopic morphology to histology and histopathology. He was undoubtedly a person with a powerful vision of the future, as demonstrated by his close association with the physicist and naturalist Giovanbattista Amici (1786-1863) who introduced the immersion objective to microscopy. The activities of Alessandri were so intense that he had little time for private life. Later, due to an infection, his right hand was amputated by Francesco Rizzoli, and his colleagues had expressed the desire to conserve it in the Museum of Pathology. This should not be shocking, however, as the skeleton of Cesare Lombroso is still housed there. Sur-
prisingly, Antonio Alessandrini did find some time for politics and was part of the triumvirate that organized the Bolognese resistance to the Austrians, which ended on May 17, 1849, after four days of bombing.

The founding of the Society of Medical Surgeons also played an important role in anatomical pathology (1823), which encouraged the presentation of autopsy cases during its gatherings. The cases were published, and the removed organs were destined, together with an accurate description, to the Museum of Pathology which became their perpetual custodian (many are still there today!).

The moon on the insignia: Cesare Taruffi

In 1844 Cesare Taruffi, then a young surgeon, was nominated as temporary anatomist and curator of the Museum of Pathology, and after a brief time became the first Professor of Anatomical Pathology at Bologna (Fig. 3). Taruffi, who came from an important and historic family, was born in Bologna on March 23, 1821. Curiously, for our analogy, Taruffi always wore an insignia of a crescent moon that was later reproduced by the Society of Medical Surgeons in Bologna over which he presided, in addition to being the Dean of Medicine.

He initially dedicated himself to surgery and was a pupil of Francesco Rizzoli, founder of the Orthopaedic Institute at Bologna that carries his name. From the beginning, however, he had a great interest in anatomical pathology. Soon thereafter, he also became involved in politics, and documents at the archives of the Museum of the Risorgimento in Bologna show that on November 6, 1847 he was nominated second lieutenant of the third battalion of the civil protection unit that had just been created by Pio IX. What came next is less clear, and the precise role of Taruffi in the war from 1848–49 is not known with certainty. A letter dated April 30, 1848, signed by Carlo Bignami, one of the defenders of Venice after the fall of Vicenza and the surrender of the volunteers of the Vatican on June 11, nominated him as surgeon of the first battalion destined for military operations in the Veneto region to fight against the Austrians. Another letter, dated June 1, was from Padua close to where the Bolognese volunteers were fighting the Austrians. Successive documents showed that he had an intense activity in Venetian hospitals, where he stayed from June 30, 1848 until January of 1849 before being recalled to Bologna for the planning of a military hospital. Even if fragmentary, the records of his activities lead one to believe that Taruffi was united with the volunteers of Bignami after the surrender on June 11.

Information about what happened later is even more scarce. A letter from the Minister of War of the Roman Republic dated June 1, 1849 nominated him as Surgeon General of the Bologna Legion, although it is not clear what his precise role actually was. From extradition papers of the command of the French army, on July 7 it was apparent that he was in Rome until the fall of the Roman Republic on July 4, 1849. Nonetheless, Taruffi was not identified as particularly dangerous as he was able to leave the city armed and in uniform, in accordance with the conciliatory strategy initially adopted by the French. The large number of marks and stamps on his extradition papers indicate that his movements towards Bologna were left largely undisturbed by both the Republicans, who were marching with Garibaldi, and the Vatican Authorities. There is no tangible proof that Taruffi carried out any patriotic activities in either Venice or Rome. Likewise, there is no evidence that his career suffered any impediments from the Vatican or from his relationship with the liberal group in Bologna, and in particular with Marco Minghetti.

Without doubt, the numerous positions in healthcare held by Taruffi, conferred by the government in Bologna in autumn of 1859, demonstrate that he had the complete trust of the liberals: this is in agreement with the moderate stance held by Taruffi during the revolt of 1848–49, and in concordance with the criticisms of the Republicans by Luigi Carlo Farini, the physician and politician from the region that helped join the Emilia and Romagna areas, and also instituted the Department of Anatomic Pathology.
In any case, it wouldn’t seem correct to attribute the academic career of Taruffi to his patriotic merits. The enormous amount of work carried out by Taruffi until his death in 1902 demonstrates that he had an excellent knowledge of anatomic pathology. In addition to creating a new institution from practically nothing, he dedicated a large part of his activities to teratology. His work in this area was incredibly tenacious, and he collected a massive amount of materials that were collected in the museum, which in 1859 was renamed the Museum of Anatomic Pathology. He conserved foetuses and malformed infants in alcohol, and he also commissioned reproductions in wax and other materials.

Much of the material he collected for his studies on teratology was left to him by other scholars. After 1863, in fact, many human specimens previously conserved by veterinarians, for the most part the illustrious morphologist and patriot Antonio Alessandrinì, were bought by the Museum of Anatomic Pathology. At the same time, Francesco Rizzoli gave Taruffi a large number of gynecological specimens, which he had collected as head of the Obstetrics Clinic during which time he also carried out a number of particularly audacious interventions. The most faithful teratological preparations in Bologna were the work of the anatomist Luigi Calori together with Cesare Bettini, a famous wax sculptor and anatomic designer; having examined the internal organs and described the malformations, Calori conserved the skeletons and internal organs that were then dried and injected using a sophisticated technique.

Calori is an excellent example of the intellectuals in the 1800s who studied natural science and morphology. He had the capacity to prepare specimens with great efficiency, which is demonstrated by the fact that many are still preserved 200 years later. He made even greater contributions through his scientific publications, which were always illustrated with figures drawn with great precision. When reorganizing the museum, it was noted that Cesare Bettini created his illustrations with such rigour that there was little difference from the original specimen. In fact, many experts were astounded by the impressive similarity between the original and the illustrations, already evident from the publications of Rodati. During the reorganization of the Museum of Anatomy at Bologna (which is now part of the Museum of Anatomic Pathology), we highlighted the close relationship between specimens present in the museum and their corresponding illustration, and how publications dating to the 1800s can allow the recovery of specimens whose original purpose was forgotten.

In addition to teratological publications, Taruffi also left a monumental composition, *Storia della teratologia*. This masterpiece abounds with historical information and erudition; it is also notable for the enormous effort needed to complete such a complex volume. This great work of Taruffi is still known abroad, even though it has been largely forgotten in Italy and Bologna. Taruffi did not finish what he had set out to do: the publication of *Storia della teratologia* began in 1881 and was finished in 1894 when he was old and in bad health. His intent was, however, to complete an even larger systematic monograph, which was left unfinished when he died in 1902.

We know little about his abilities as a teacher, although he did author an essential text on anatomic pathology in 1870 in which it is apparent that he was well versed with the relevant topics in Europe, namely those of Virchow. Certainly this was a point in his favour as the Vatican Government had ceased its control 10 years earlier, and Taruffi was already able to take part in a more European atmosphere.

Cesare Taruffi died in July 1902, and Frank Gonzalez-Crussi dedicated a large part of his famous volume to him, as well as another work on conservation of the human body, which was in part inspired by the Museum of Anatomic Pathology in Bologna. Taruffi’s fame as a classifier of malformations was merited. For years there was talk of a volume in German by Taruffi at the library of the University of Heidelberg, which would have been the third and last part (incomplete) of his classification of teratology. Contemporary progresses in morphology and collection of epidemiological data prompted Taruffi to greatly enlarge his original outline for *Storia della teratologia*, and he wrote several articles on the subject.

Even at an advanced age, Taruffi appeared to be extremely coherent and accurate, and was perturbed by a problem that had troubled the West from the 1700s until the 1900s: the determination of gender and the existence or negation of a true confine between men and women. Along these lines, it was the psychological aspects of carriers of malformed sex organs that led Taruffi to study some of the most disturbing themes of the late 1800s: homosexuality, bisexuality and pedophilia.

The French revolution led to a strong exaltation of virility, in contrast to the attribution of a maternal-family role of women, and the 1800s were transformed into a battleground between a patriarchal role and emancipation for women. A typical example of this conflict in Italy is the life of Giuseppina Cattani. Such conflicts, together with the discoveries on the complexities of gender determination by embryologists and the realization of the extent of homosexuality in Western society, made for very interesting studies in sexology.

It was in this context that the works of Taruffi were translated into German, which demonstrated that in spite of his age, he was particularly up-to-date on the problem and among the most well-known specialists at that time. Most likely, at the beginning of the 1900s, Germany
was the most open and lively culture in the world and may be considered the cradle of sexology. It is not surprising, therefore, that a volume that was so rich in information, such as that by Taruffi, was translated into German. Likewise, it is not surprising that Sigmund Freud (1856-1939) was interested in Taruffi’s work. At the beginning of the 1900s, Freud became aware of the close relationship between psychological affections and ‘anomalies’ in sexual behaviour. The search for a physical explanation of ‘abnormal’ sexual behaviour led Freud to the works of Taruffi, and in fact Taruffi was cited at the beginning of Freud’s three publications on sexual doctrine. For Freud, morphology was a critical aspect, and he demonstrated the indistinctness of embryological development on gender, contributing to the Freudian revolution. The dynamics of the determination of gender also led Freud to see a type of embryogenesis in the development of a psyche, for example in the discovery of infant sexuality and its development. Taruffi was, albeit involuntarily, a sort of detonator that led to an explosion in the research activities of Freud, which characterized his work from 1905 to 1910 and radically changed the way we think.

Little is known about the private life of Taruffi. He was widowed at a young age, and did not remarry. He was a fervent polemicist, but nothing in his writings would lead one to think that he was a bizarre person. One recently solved enigma was the discovery that many at Bologna had confused Taruffi with the oculist Tartuferi (with the accent on the ‘u’: strangely, Taruffi is known in Austria as Tartuffi), founder of the oculist clinic in S. Orsola during the war in Libya. Tartuferi was perhaps a bit unconventional: he had a great fear of thieves and swindlers and had transformed his villa in the hills of Bologna into a sort of fortress, with windows that opened by themselves in a haphazard manner, allowing him to shoot upon eventual robbers. Interestingly, such a model was later adopted for the fortifications on the Maginot line.

The moon rises ... and the amount of work increases

When Taruffi retired from teaching in 1893, Giovanni Martinotti (1857-1928) took his place in Bologna at the peak of his academic career which started in Turin (Fig. 4). From 1889 to 1891 he was the head of anatomic pathology at the University of Modena, where he began an intense interest in microbiology, then a branch of anatomic pathology. From 1891 until he arrived at Bologna, he was at Siena. At Bologna, he dedicated a large part of his time to teaching. Along these lines, he was passionate about the creation of a new institute of anatomic pathology, the present institute in Via Irnerio, which was completed in 1907. The new institute combined both anatomic pathology and normal anatomy. The previous institute was crumbling, and was located in Palazzo Malvezzi Lupari, and even the museum was restored. From the time of Martinotti there were still several autopsy registers that were later transferred to the institute of normal anatomy. In later years under the direction of Martinotti, autopsies were officially and definitively the responsibility of pathologists. This led to a large increase in the number of autopsies performed (from 100 to almost 1000 per year).

During the same period, the first histological diagnoses were carried out on biopsies and surgical specimens. Histopathologic techniques were a major interest for Martinotti, probably because at that time diagnostic biopsies from malignant neoplasms had just begun to have practical relevance. In our opinion, it was Martinotti that first began to collaborate with clinicians and radiologists to systematically study malignant neoplasms. This supposition is demonstrated by statistical studies started by Vigi that continued until Armando Businco was no longer head of the department.

Martinotti published many articles, but his work in anatomic pathology was almost never particularly exciting, with one exception to be considered later. One reason for this was perhaps that Martinotti was more interested in microbiology, and in particular on finding an antituberculosis vaccine. He claimed that his vaccine provided excellent protection, but in reality it worked only in cases that were not severe, and it couldn’t eliminate recurrences. Apart from the tangible results, however, his research demonstrated a notable understanding of tuberculosis and the problems associated with it. Curiously, there is now a renewed interest in his work, probably linked to the increased incidence of tuberculosis due to chemoresistance and the poor efficacy of...
the BCG vaccine. Bindo De Vecchi brought to light that it was not Giovanni Martinotti, in contrast to what had been previously reported by many, who discovered the cortical neurons called “Marinotti cells”: their real discoverer was a pupil of Camillo Golgi, namely Carlo Martinotti 52,53.

Martinotti died suddenly and tragically, and the arrival of Giulio Tarozzi from Turin (1868-1948) as head of the department was somewhat unexpected. Before discussing Tarozzi, however, an extraordinary event should be described that can be compared to the passage of a comet: the period of Bindo De Vecchi.

The years of Halley’s comet: Bindo De Vecchi (Fig. 5)

Fig. 5. Bindo De Vecchi.

As a young and brilliant student of medicine, De Vecchi made a great impression on the famed clinician Augusto Murri, who directed him towards anatomic pathology. Even if De Vecchi did not reach the height of his career at Bologna, he still maintained a solid relationship with Martinotti, and even wrote his eulogy. This latter event was extremely important since it was the only publication that did not consider him to be the discoverer of Martinotti cells, in contrast to later historians 54.

De Vecchi as a talented morphologist. In 1903, he diagnosed a myocardial infarct in an autopsy report (now in the Institute of Anatomy). This was not a trivial insight since most believe that the anatomical concepts behind myocardial infarct were elaborated in the United States after the First World War. In reality, the correlation between myocardial necrosis and occlusion of a coronary vessel had already been clearly stabilized during the second half of the 1800s by Cohnheim 14, whose publications were in the library of Giovanni Martinotti. His abilities as a morphologist are also evident in the area of neoplastic pathologies. De Vecchi had a perspective that was very modern for his time, and more like that of a surgical pathologist, and one of his reports on oncology was published in English in a medical journal in New York 55. His knowledge of English and his interest in American research was unusual at the beginning of the 1900s 47. For example, De Vecchi, in 1908, preferred the school of Schmorl at Dresden, to deepen his knowledge. His interest in studies carried out in the United States thus makes him person of noteworthy foresight, similar to Vittorio Putti, the well-known orthopaedist who gave worldwide fame to the Rizzoli institute in Bologna.

De Vecchi was the son of an army officer, to which his intense sense of patriotism can be attributed. It is not known, however, if this fact can adequately explain his restless existence. To us, it seems that De Vecchi was a generous person in both public and private life, and was admired by his students. It should be remembered that he volunteered for the First World War, provided medical aid to the victims of the earthquake in Messina and Calabria in 1909 and was intensely involved in the fight against cholera in Syracuse (1911). This was a very successful time for De Vecchi, and he was awarded a medal of honour in the war and promoted to a high-ranking medical officer.

His professionalism as a pathologist should not be forgotten during wartime, and he published a report in 1919 in which he presented death statistics among draftees after the war had ended (Autumn 1918) 56 on the influenza epidemic. De Vecchi and his colleagues were surprised by the severity of the pulmonary damage seen in most victims. They were also aware that they were facing a disease that gave rise to extensive pulmonary symptoms with unusual morphology. They did not characterize it as acute interstitial pneumonia, and adhered to the common idea that it was caused by bacteria, which led to death in more serious cases. De Vecchi is also considered a clever experimental pathologist, and his studies on Addison’s disease secondary to tuberculosis of the adrenal glands, were published in various journals as well as in the Medical News 57.

Lastly, there was the spectacular academic career of De Vecchi. He headed the first anatomic pathology department in Perugia in 1920 (a sort of recovery after he left as a war volunteer in 1915), then at Palermo, and finally at the Medical School in Florence where he succeeded Guido Banti (1925). In Florence, De Vecchi reached the apex of his fame and became President of the university; when he died in 1936, he was celebrated as a national hero. He was a fervent fascist, which today we can only talk about, but cannot fully understand. One thing however is certain: De Vecchi wasn’t just anyone, but was a feather in the cap of Mussolini, along with Guglielmo Marconi.
Approaching the full moon: Giulio Tarozzi

While Taruffì is remembered, thanks to the Museum, and Martinotti, thanks to a commemorative plaque in the main lecture hall of the Institute, at least a small remembrance is still present, Tarozzi has been largely forgotten. The only traces we found were a commemorative text written by one of his students and successor at Bologna: Armando Businco. The commemoration was a very precise appraisal of the scientific activity of Tarozzi, who published numerous papers in German and English, especially during the period before his arrival in Bologna, when he was in Pisa, Siena, Cagliari and Modena. Nonetheless, his assessment did not allow for an understanding of the man during his most active period, when he should have been at the peak of his productivity. Tarozzi was nonetheless a good morphologist, and conformed to the German school as demonstrated by his very last studies on postencephalitic Parkinsonism.

During his last years, Tarozzi seemed fascinated with philosophical problems related to the meaning of life and the mysterious world that surrounds us. We know from one of his brothers that Tarozzi had been a professor of philosophy, which had a profound influence on him. Evidently, however, Giulio Tarozzi lived an autonomous, lively life in his philosophical undertakings. Recently, in fact, Sandra Linguerrì and Raffaella Simili discovered that he was part of a group of Bolognese scholars that had invited Einstein to Bologna for a series of lectures. The group was among the minority in the country that still had relations with Einstein after he was publicly condemned by Italy following its adhesion to the racist politics of Hitler.

Similar to Martinotti, Tarozzi also carried out microbiological studies and was particularly interested in culture medium for anaerobic bacteria. When the Museum was transferred at the turn of the millennium, several autopsy specimens were found that dated to Tarozzi’s time. Probably, it is not by chance that the specimens were from neoplastic pathologies that were intensely studied by his colleagues. A series of smears without cover slips were completely unexpected, which were specimens of tetanus and anthrax, and still in an optimal state of conservation. Undoubtedly, these were among the last traces of the microbiological collection of Giulio Tarozzi. According to Mario Alberto Dina, the only surviving pathologist that knew him personally, these pathogens were indeed studied by Tarozzi.

Full moon or dawn? Armando Businco

(Fig. 6)

Tarozzi retired from teaching in 1938, and his position was filled by Armando Businco (1886-1967), from Ierzu (Nuoro), the father of anatomic pathology in Bologna and of a generation of pathologists that are still alive. Businco began his studies in Cagliari, but they were soon interrupted by the war when he volunteered in 1916 as a medical officer. He was extremely active during that period in revising histological samples on the effects of toxins on animals obtained by experiments commissioned by the Minister of War. The study predicted the massive use of poisons in the First World War. Businco began the histopathological revision when the use of chemicals on humans had just begun, and he was thus able to compare experimental data with that on human victims. Several publications dating to 1920 were also published, one of which was in English, which demonstrate the attention he gave to damage induced by inhaled substances on the alveolocapillary membrane in the lung.

After the war, Businco taught anatomic pathology at several universities including Perugia, Cagliari and Palermo, until he returned to Bologna in 1938 where he remained until 1956. His arrival in Bologna corresponded to the height of his national fame, and he was well known for his studies on endemic malaria in Sardinia. He took particular interest in educating physicians on the problem of malaria and wrote L’infezione malarica, which was re-examined in the 1990s by Antonmaria Mancini and later made an obligatory text for anatomic pathology students in Bologna. Both before and after the Second World War, Businco passionately contributed to antimalarial efforts in Italy. Today, his contribution is admired to the point that Italy is often considered as a model for the eradication of malaria. It is disappointing that in recent studies on malaria Businco was not remembered.
His permanency in Bologna is connected with a dramatic and mysterious event: in 1944, Businco was arrested by the Nazis and deported, although he was able to escape and hide until the end of the war. Prior to the war, Businco did not seem to openly oppose the fascist regime. During the German occupation, however, something appeared to change, and he countered an attempt by the Germans to obtain radio equipment at the Radio Institute in Bologna. Moreover, he was also in contact with a group of students and young underground physicians that was brutally massacred. He later commemorated the group with a plaque placed in the new Institute inaugurated in 1948. The speech that Businco gave for the inauguration was published. The clear and moving demonstration of the strong sentiments in place at that time were further confirmed in a letter sent by Bartolo Nigrisoli, a professor of surgery at Bologna, who was also distanced from the University for not having pronounced his loyalty to the fascist regime.

Even after many years, the specific details of what happened to Businco at that time are still unclear. Most likely, an explanation lies in the ambiguous role that the healthcare system in Bologna had from 1943 to 1945. The Rizzoli Orthopaedics Institute and the Institute of Traumatology, headed by Oscar Scaglietti, was one of the most important military hospitals for the German retreat. While the Italian Red Cross was in contact with both the underground and the Allies, there was undoubtedly a sort of consent by the Germans. The tragedy of Businco and his medical students was perhaps an ‘anomaly’. This would explain how Businco, even if nominated president of the Commission for ‘Purging’, was not able to overcome his symbolic role after the liberation. In addition, the supposed evidence that linked the physicians in Bologna to German opposition was never followed up.

Businco was a decisive believer in the use of autopsies, and held that clinicians should personally be present in order to provide a frank exchange of ideas with pathologists. Even the directors of other institutes were invited to be present during autopsies. The complete reconstruction of the autopsy archives of the University, from 1838 to 1999, certainly presented some inconsistencies with the myth created among the pupils of the famed Sardinian pathologist. Until the 1920s, autopsies were almost exclusively performed for educational purposes. During the time of Taruffi, they were carried out only from October to June, and autopsy reports were compiled and signed by students, and revised by Taruffi and his colleagues. This activity in itself was considered a preliminary experience in order to be admitted for final exams. It was also documented that, in accordance with the tradition of Malpighi and Morgagni, clinicians personally performed autopsies on their patients, in what Juan Rosai has proposed as “posthumous analysis”. Following the birth of modern histopathology, the pathologist was involved with the clinician only when it was necessary to complete the histopathological study as a result of increasing specialization and costs related to maintaining a histological laboratory. As already mentioned, it was Virchow who suggested that autopsies be performed by pathologists due to the large increase in autopsies performed in the 1900s, which didn’t begin in Bologna until the 1920s under the direction of Martinotti. Later, the number of autopsies levelled off, until the 1960s when they began to decline in number. A disconcerting fact is that the introduction of dictation actually lowered the quality of autopsy reports, with a slow but progressive growth in the number of reports that were left outstanding. What is even more surprising is that histopathology was used only rarely for diagnosis, and often with alarming results, as for the diagnosis of bronchopneumonia.

Such a disappointing situation, in an area that was always considered vital for pathologists, was often brought into question by anatomic pathologists, starting with Martinotti, who strangely makes reference to the ‘Golden Age’ of autopsies, which perhaps with the exception of the Viennese school, probably never existed. Nonetheless, apart from autopsies, even before the war, and at the same time and perhaps independently of the Americans given the cultural barrier that was still present, Businco understood the changes that were taking place at the time in anatomic pathology. This mainly involved improvements in surgical technique that permitted a greater number of interventions needing biopsy information on organs that were previously inaccessible. For the pathologist, this was a new and challenging type of diagnostic histopathology that required precise clinical information and close collaboration with the clinician. This profound change in modern anatomic pathology was anticipated by Businco, and he inspired the idea that a new approach was needed, which he called an ‘anatomo-clinical approach to anatomic pathology’ that he introduced in 1938. His insistence was fundamental for renewing the Institute after the Second World War when other European schools were permeated with the new American concept of surgical pathology.

In Businco, the roots of the merits and deficits of anatomic pathology can be found in the identity crisis that had afflicted the discipline for some time. From the second half of the 1800s up to the 1950s, the pathologist had complete control over medical laboratories, exerting his influence over clinicians in a manner that was not always well accepted. The influence of this dominant role is evident in the writings of Businco, especially between the two World Wars, in which he had forwarded the idea that each large clinic should be under the direction of a pathologist. As for many other pathologists, Businco perceived the importance of radiology, in addition to the potential threat that radiology posed in terms of control exercised by the pathologist. For this
reason, he sustained that radiology was a morphological science, and that radio-diagnosics should be a branch of anatomic pathology. Similarly, it was his desire that radiotherapy should be a branch of pathology as it was widely used in diagnostic oncology.

Businco was fortunate enough to not experience the crisis of modern anatomic pathology. We believe that if he had lived long enough, he would probably have understood the new orientation needed for its rebirth. In our opinion, he was a person with great intuition, even during the last years of his long career. After he first came to Bologna, he began to recognize the increase in lung cancer, which until that time was relatively rare. In the last years of his career, Businco was also interested in other ‘popular’ diseases such as atherosclerosis and disease involving histocytes and the immune system. For him, lung cancer was truly worrisome. In 10 years of publications, he noticed a vast increase in the number of lung cancers, and during the 1950s he attempted a rational classification system based, as always, on accurate anatomo-clinical data. These studies led to a publication, later translated in several languages, accompanied by accurate macroscopic and histopathological observations. In our opinion, with the exception of the discovery of microcytoma by Azzopardi, little has been added to the findings of Businco until the development of molecular pathology.

A person who is so sensitive to moments of crisis in medicine would have been able to guide Italian pathologists towards a necessary self-criticism, by promoting the theories and teachings of anatomic pathology already present in professional activities: the necessity of transforming anatomic pathology into a subject that, as in all laboratory medicine, is not the foremost contributor, but a discipline that is principally dedicated to clinical benefits.

References
7 Meshberger FL. An interpretation of Michelangelo’s creation of Adam based on neuroanatomy. JAMA 1990;264:1837-41.
18 Rodati L. In praeparationes osseae musei pathologici bononiosis animadversiones. Opuscoli Scientifici 1818;II:362-83.
19 Rodati L. In praeparationes myo-pathologicas musei pathologici bononiosis animadversiones. Opuscoli Scientifici 1819;III:397-404.
20 Rodati L. In praeparationes splancho-pathologicas musei pathologici bononiosis animadversiones. Opuscoli Scientifici 1823;IV:362-78.
Dalla luna nuova al plenilunio: l’Anatomia Patologica bolognese dal Cinquecento ad Armando Businco

P. SCARANI**, V. EUSEBI*

*”Marcello Malpighi”; **Museo delle cere “Luigi Cattaneo”, Università di Bologna, Italia

Antonmaria Mancini (1929-2007) celebrò il 12 Giugno 1989 i 130 anni dell’istituzione della I Cattedra di Anatomia Patologica dell’Ateneo Bolognese.


Antonmaria Mancini era così. Diceva spesso: “Se Dio ha reso noi uomini liberi di fare anche il male, io devo per forza fare altrettanto coi miei collaboratori”.

Luna nuova: morfologia nel Cinquecento bolognese

Senza risultati pratici sulla salute del malato, la medicina non può godere di grande reputazione fra la gente. Questo stato d’animo poco caritatevole del grande pubblico verso i ricercatori è ben evidente sino alla fine della seconda guerra mondiale. Le grandi scoperte di Koch a fine Ottocento, per esempio, lasciavano un senso d’amaro in bocca, perché, a causa della consistenza ancora scarsa della farmacologia, nonostante le eccellenti conoscenze sulle malattie, la terapia aveva ancora un’efficacia che si discostava di poco da quella dei guaritori e dei ciarlatani. Soltanto la Seconda guerra mondiale “fece sorgere il sole” dell’ottimismo, oramai pronto ad affermarsi dopo un trentennio di successi terapeutici finalmente tangibili.

Giulio Cesare Aranzi (1530-1589)

Aranzi (Aranzio è la forma latinizzata), autentico bolognese (Fig. 1). Scoprì la circolazione fetale e la descrisse in modo splendido, con un latino da antologia. L’unica traccia che persiste di questi studi è il dotto venoso dell’Aranzio. Aranzi inoltre scoprì l’ippocampo, che lui,
più familiarmen
ti chiamava ‘baco da seta’. Forse, que-

st’immagine, a Bologna era recepita molto più facilmente,
rispetto a quella del cavalluccio marino, dato il grande
sviluppo locale dell’industria della seta. Tale scoperta fu
una rivoluzione per le neuroscienze. Individuando l’ippo-
campo, Aranzi si accorse infatti dell’esistenza di un corno
in più (il corno temporale) del ventricolo laterale, ignoto
anche al suo maestro Vesalio. La scoperta è straordinaria,
anche alla luce delle gravi difficoltà che, prima dell’Otto-
cento, epoca dello sviluppo dei fissativi, s’incontravano
nello studio macroscopico dell’encefalo. Lo dimostra la
scarsa chiarezza delle tavole vesaliane che illustrano il
sistema nervoso nell’edizione del De humani corporis fa-
brica del 1543. A questo proposito, non riusciamo ancora
capitarci come abbiano potuto attribuire a Michelan-
gelo (1512) la rappresentazione d’un cervello umano nel
gruppo di Dio circondato da creature volanti nella creazio-
ne di Adamo della Cappella Sistina. Ciò, si badi bene, fon-
dandosi su confronti, non con tavole dell’epoca, ma con
quelle novecentesche di Netter ⁷. Oltre alla individuazione
di strutture anatomiche, Aranzi pubblicò la prima opera
anatomo-patologica Bolognese che combinava l’anatomia
con la descrizione di processi patologici.

Un’altra opera dello stesso autore, edita a Venezia nello
stesso anno, insieme con gli studi anatomici: De tumo-
ribus secundum locos affectos ⁸, consiste in una raccolta
eterogenea (idrocefali, cisti, ascessi, masse solide) di tu-
mefazioni (tumori, appunto), suddivise per sede. Sono il
frutto evidente dell’attività d’un medico pratico, dedito,
fra l’altro, anche alla chirurgia.

Oggi, quasi nessuno ricorda Aranzi a Bologna.

Nella medicina bolognese del Cinquecento c’era davvero
un buio da luna nuova, se si considera che la figura do-
minante nell’università era Gerolamo Cardano ⁹. Gli stu-
denti preferivano, comunque, Ulisse Aldrovandi (1522-
1605) ⁹. Questi era naturalista, più che medico. Aveva
tuttavia una visione così moderna della morfologia, da
studiarla con cura anche gli organi interni degli animali.
I suoi studi teratologici, poi, sono di straordinario rigore.
Anche Aldrovandi non ebbe grande successo a Bologna,
nonostante la reputazione internazionale, tuttora conser-
vata. Riuscì a dare alle stampe solo una minima parte
delle sue opere. Neppure lo volle aiutare suo cugino, il
Papa bolognese Gregorio XIII (quello della riforma del
calendar) che pure aveva tratto grandi vantaggi, anche
pratici, dall’opera scientifica aldrovandiana ¹⁰.

Non possiamo qui fare a meno di ricordare che il pro-
simo grande precursore bolognese dell’anatomia pato-
logica, Marcello Malpighi, si formò come curatore delle
grandi collezioni naturalistiche di Aldrovandi.

**Falce di luna crescente**

*Marcello Malpighi* (1628-1694) e i suoi discepoli.

Malpighi, da Crevalcore (Bologna), fu allo stesso tempo
un grande anatomico, anatomo-patologo e clinico (Fig.
2). Seppe per primo applicare la microscopia in grande
stile allo studio della struttura degli organi. Per questo,
ancora oggi si ritrova molto spesso il suo nome associa-
to a parti della struttura del rene, della milza e della cute.
Sono di grande pregio anche i suoi studi sul polmone e
sullo sviluppo dell’embrione. Malpighi deve però anche
essere considerato un anatomo-patologo perché eseguiva
accurate autopsie corredate da notizie cliniche. I referti
sono preceduti oltre che da riferimenti anagrafici anche
da dettagliate storie cliniche. Il suo metodo autoptico
era simile a quello dei nostri giorni. Si procedeva con
l’osservazione del cadavere e quindi si procedeva al-

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**Fig. 1. Giulio Cesare Aranzi (da Brambilla Giovanni Alessandro.
Storia delle scoperte fisico-medico-anatomico-chirurgiche fatte
dagli uomini illustri italiani. Milano 1782, Edizione anastatica Bolo-
gna: Arnaldo Forni 1977, p. 188).**

**Fig. 2. Marcello Malpighi (quadro di Carlo Cignani, Galleria Bor-
ghese, Roma).**


Verso la luna piena: da Napoleone alla Repubblica romana


Grande importanza per l’anatomia patologica ebbe anche la fondazione della Società medica chirurgica bolognese (1823), la quale si fece promotrice della presentazione di casi autoptici nelle proprie sessioni. I casi dovevano essere pubblicati, e gli organi prelevati erano destinati, insieme con un’accurata descrizione, al museo di patologia, che ne diveniva il custode perpetuo (in molti casi così è stato fino ad oggi!).

La luna nell’emblema: Cesare Taruffi

Nel 1844 fu nominato settore anatomico temporaneo e curatore del museo il giovane chirurgo Cesare Taruffi, destinato a divenire entro breve tempo il primo professore di anatomia patologica dell’Ateneo bolognese (Fig. 3). Taruffi, discendente da un’antichissima famiglia, cui appartennero numerosi illustri personaggi vissuti sia a Bologna che in Toscana, nacque a Bologna il 23 marzo 1821. Curiosamente per la nostra storia, l’emblema dei Taruffi porta un crescente in capo. Esso è riprodotto nella sede della società medica chirurgica di Bologna, in quanto il nostro ne fu a lungo presidente, oltre che preside della facoltà medica. Si dedicò inizialmente alla chirurgia e fu allievo di Francesco Rizzoli, il fondatore dell’omonimo Istituto ortopedico. Già dagli esordi, però, si curava anche dell’anatomia patologica, come è dimostrato dalla sua qualifica di settore anatomico. Ben presto si trovò coinvolto nella politica. Da documenti raccolti nell’archivio del Museo del Risorgimento di Bologna, risulta infatti nominato, il 6 novembre 1847, sottotenente del terzo battaglione della guardia civica, da poco istituita da Pio IX. Le notizie successive da noi reperite sono frammentarie, e non fanno intendere bene il preciso ruolo avuto dal Taruffi nelle guerre del 1848-49.

Una lettera del 30 aprile 1848, firmata da Carlo Bignami, uno dei difensori di Venezia dopo la caduta di Vicenza e la resa dei volontari pontifici dell’11 giugno, lo nomina medico chirurgo del primo battaglione mobile civico, destinato alle operazioni militari nel Veneto, contro gli Austriaci. Un’altra lettera del 7 giugno, ce lo fa trovare a Padova, poco lontano, quindi, dai teatri dei combattimenti dei volontari bolognesi contro gli Austriaci provenienti dalla Carnia, che tentavano di congiungersi al Radetzky. Lettere successive testimoniano un’intensa attività del Taruffi negli ospedali di Venezia, dove già si trova il 30 giugno, fino al gennaio del ’49, allorché è richiamato a Bologna per la progettazione di un ospedale militare. Queste notizie, pur frammentarie, fanno pensare che il Taruffi si sia unito ai volontari del Bignami, dopo le resa dell’11 giugno. Le notizie successive sono ancora più scarse. Una lettera del Ministero della Guerra della Repubblica Romana, datata 1 giugno 1849, lo nomina chirurgo maggiore della legione bolognese. Non è chiaro però quale sia stato il suo ruolo preciso nella difesa di quella Repubblica. Da un foglio di via, che gli rilasciò il comando dell’esercito di occupazione francese il 7 luglio, risulta evidente che egli si trovava a Roma fino a dopo la caduta della Repubblica (4 luglio 1849). Evidentemente il Taruffi non si era però segnalato come individuo particolarmente pericoloso, perché poté uscire dalla città arma-

Le più accurate preparazioni teratologiche bolognesi sono il frutto degli studi dell’anatomico Luigi Calori. Furono allestite da Cesare Bettini, famoso ceroplasta e disegnatore anatomico suo contemporaneo; esaminati gli organi interni e descritte le malformazioni, Calori ne conservava gli scheletri, e gli organi interni, essiccati ed innestati con una tecnica mirabile. Calori ci fornisce un esempio tipico delle tecniche d’uno studioso di scienze naturali, soprattutto morfologiche, dell’Ottocento. Egli aveva la capacità di raccogliere preparati (naturali o in copia) con grande efficienza. L’efficienza è evidenziata dal fatto che molti di quei preparati resistono ancora alla distanza di quasi due secoli. Il fatto di maggior rilevanza è tuttavia costituito dalle pubblicazioni scientifiche, sempre illustrate con numerose tavole che rappresentano con grande precisione i temi trattati. Per la teratologia, anzi, le tavole sono state di estrema importanza nel riordino del museo. Di solito, infatti, Cesare Bettini allestiva sia i preparati che le tavole illustrate con tale rigorosità, che, non vi erano differenze fra il preparato e l’illustrazione. Sin dal l’inizio del riordino del museo di anatomia patologica, molti esperti rimasero sbalorditi per questa spettacolare corrispondenza fra preparati e pubblicazioni scientifiche, già evidenti nelle pubblicazioni dei Rodati. Nella successiva riorganizzazione del museo di anatomia (di cui ora fa parte anche il museo di anatomia patologica), evidenziavamo come questa relazione tra museo e pubblicazioni è diffusa nei musei universitari bolognesi, e che le pubblicazioni ottocentesche permettono spesso di recuperare preparati il cui significato era andato del tutto perduto.

Oltre a varie memorie e pubblicazioni su singoli reperti teratologici, Taruffi lasciò un’opera monumentale, la Storia della teratologia. Essa corrisponde senz’altro al titolo, perché è ricchissima di nozioni storiche e di erudizione; è anche però di notevole rilievo lo sforzo gigantesco che l’autore ha compiuto per dare organicità e sistematicità a questa materia complessa. Questa grande opera di Taruffi vive ancora all’estero, nonostante che in Italia ed a Bologna sia stata dimenticata.

Per quanto riguarda il museo di anatomia patologica, Taruffi non raggiunse gli scopi che si era prefissato: la pubblicazione della Storia della teratologia fu iniziata nel 1881 e terminata nel 1894, quando egli era oramai vecchio e di salute cagionevole; il suo intento era però quello di portare a compimento un’opera ben più vasta e sistemata. Tale efficienza è evidenziata dal fatto che molti di quei preparati resistono ancora alla distanza di quasi due secoli. Nell’inizio del riordino del museo di anatomia patologica, numerose tavole che rappresentano con grande precisione i temi trattati. Per la teratologia, anzi, le tavole sono state di estrema importanza nel riordino del museo. Di solito, infatti, Cesare Bettini allestiva sia i preparati che le tavole illustrate con tale rigorosità, che, non vi erano differenze fra il preparato e l’illustrazione. Sin dal l’inizio del riordino del museo di anatomia patologica, molti esperti rimasero sbalorditi per questa spettacolare corrispondenza fra preparati e pubblicazioni scientifiche, già evidenti nelle pubblicazioni dei Rodati. Nella successiva riorganizzazione del museo di anatomia (di cui ora fa parte anche il museo di anatomia patologica), evidenziavamo come questa relazione tra museo e pubblicazioni è diffusa nei musei universitari bolognesi, e che le pubblicazioni ottocentesche permettono spesso di recuperare preparati il cui significato era andato del tutto perduto.

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La rivoluzione francese comportò una forte esaltazione della virilità, in contrasto con l’attribuzione di un ruolo materno-familiare alle donne, e l’Ottocento si trasformò in un campo di battaglia fra questo concetto patriarcale e i nascenti movimenti per l’emanzipazione delle donne. Un esempio tipico di questa conflittualità è in Italia costituito dalla vita di Giuseppina Cattani. Tale conflittualità, insieme con le scoperte sulla complessa determinazione del sesso, da parte degli embriologi, e delle reali dimensioni della presenza degli omosessuali nella società occidentale, indussero un vivissimo interesse per gli studi sulla sessuologia.

Proprio in questo contesto debbono essere considerati i saggi di Taruffi tradotti in tedesco. Egli si dimostra, nonostante l’età, particolarmente aggiornato sul problema e sui più noti specialisti del tempo. La Germania del primo Novecento è probabilmente il centro culturale più aperto e vivace del mondo, ed è la culla della sessuologia. Non sorprende, quindi, la traduzione in tedesco di un’opera così ricca d’informazioni come quella del Taruffi. Neppure sorprende che Sigmund Freud (1856-1939) se ne interessi. All’inizio del Novecento, Freud si è oramai reso conto degli stretti legami fra tante affezioni della psiche da lui studiate e varie “anomalie” della vita sessuale. La ricerca di una spiegazione fisica dei comportamenti sessuali “anormali” lo porta al saggio in tedesco di Taruffi. Questo è infatti citato all’inizio dei suoi tre saggi sulla dottrina sessuale. La morfologia è, per Freud, d’importanza critica. Essa infatti dimostra l’ambiguità dello sviluppo embriologico del sesso, e contribuisce alla “rivoluzione freudiana”. Il dinamismo della determinazione del sesso, porta infatti Freud a vedere anche nello sviluppo della psiche una specie di embriogenesi, di preistoria. Si è attuata, insomma, la scoperta della sessualità infantile e del suo sviluppo.


La luna cresce … e il lavoro aumenta

Quando, nel 1893, Taruffi si ritirò dall’insegnamento, gli subentrò Giovanni Martinotti (1857-1928), piemontese, il quale giunse a Bologna quando oramai si trovava all’apice della propria carriera accademica, iniziata a Torino (Fig. 4). Dal 1889 al 1911 tenne la cattedra di anatomi patologica dell’Università di Modena, dove si manifestò la sua propensione per la micro-biologia, allora una branca dell’anatomia patologica. Dal 1891 sino alla chiamata a Bologna, fu a Siena. A Bologna si dedicò soprattutto alla didattica. A questo proposito, fu un appassionato sostenitore della creazione del nuovo Istituto di anatomia patologica, l’attuale Istituto anatomico di via Imerio, ultimato nel 1907. In esso erano accolti sia l’Istituto di anatomia patologica che quello di anatomia normale. La sede precedente era ormai fatiscente: si trattava infatti ancora di quella di Palazzo Malvezzi Lupari, a un passo dal Rettorato. Anche il Mu-
De Vecchi, giovane, brillante studente di medicina, impressionò profondamente il grande clinico Augusto Murri. Questi, con notevole perspicacia, lo indirizzò all’anatomia patologica, presentandolo al Martinotti. Pur non essendo destinato a raggiungere, come vedremo, l’apice della carriera a Bologna, De Vecchi conservò sempre un saldo legame con la città. De Vecchi preferirà la scuola di Schmorl a Dresda, per l’approfondimento della propria preparazione.

Gli anni della cometa di Halley: Bindo De Vecchi (Fig. 5)

Fig. 5. Bindo De Vecchi.
La vicinanza alla cultura statunitense ce lo presenta, quindi, come uomo dotato di notevole lungimiranza. Simile a lui fu Vittorio Putti, il grande ortopedico bolonese, che seppe dare rinomanza genuinamente mondiale all’Istituto Rizzoli di Bologna.

De Vecchi era figlio di un ufficiale dell’esercito. A ciò è attribuito il suo particolare senso patriottico, che lo fece assicurare ad una dignità politica di grande rilevanza. Non sappiamo se questa sia una spiegazione adeguata di tutta la sua inquieta esistenza. A noi sembra che De Vecchi fosse un generoso, sia nel lavoro, dove era molto amato dagli allievi, che nella vita pubblica. A questo proposito, a parte il volontariato nella prima guerra mondiale, bisogna ricordare il soccorso prestato ai terremotati di Messina e Reggio Calabria del 1909, e l’impegno profuso per la lotta al colera nel siracusano (1911). Il periodo bellico fu molto celebrato, per l’eroismo dimostrato dal De Vecchi, che gli valse la croce di guerra ed il conseguimento del grado di ufficiale medico superiore. Non si può tuttavia dimenticare la sua professionalità di patologo, anche in guerra. Possiamo, a questo proposito, citare una sua pubblicazione del 1919, in cui presenta una statistica delle morti fra militari di leva nel periodo attorno alla cessazione del conflitto (autunno 1918) 56. Si tratta della grave epidemia influenzale di quell’epoca, la famosa ‘spagnola’. De Vecchi ed i suoi collaboratori furono sorpresi dalla gravità della malattia polmonare riscontrata in gran parte delle vittime. Si accorgono inoltre di trovarsi di fronte ad un quadro polmonitico molto esteso, e con morfologia insolita. Se non arrivano a caratterizzarlo come una polmonite interstiziale acuta, si discostano tuttavia dal pregiudizio comune che per lungo tempo volle come esclusivamente da cause batteriche le polmoniti che portano a morte i malati più gravi nel corso di pandemie influenzali particolarmente severe.

De Vecchi è anche considerato un patologo sperimentale di grande abilità. Particolarmente celebrati furono gli studi sulla malattia di Addison secondaria a tubercolosi dei surreni, pubblicati in varie riviste, ed ancora una volta nelle Medical News 57.


### Luna quasi piena: Giulio Tarozzi


Negli ultimi anni il Tarozzi sembrava spesso affascinato da problemi profondi, coinvolgenti il significato della vita e del mondo misterioso che ci circonda. Sapevamo che un fratello di Tarozzi era stato professore di filosofia, e che aveva avuto molta influenza su di lui, soprattutto introducendolo alle speculazioni dei filosofi della scienza dell’epoca. Evidentemente, però, Giulio Tarozzi aveva una vita autonoma molto vivace, in questa attività speculativa. Recentemente, infatti, Sandra Linguerr e Raffaella Simili hanno dimostrato che egli faceva parte del gruppo di studiosi bolognesi che sostenevano Einstein, e l’invitarono anche per alcune conferenze nella nostra città 61. Essi poi si distinsero per essere tra i pochi Italiani ancora in rapporto con lui, dopo che Einstein ebbe pubblicamente osteggiato l’Italia per l’adesione alla politica razziale di Hitler.

Come Martinotti, anche Tarozzi compì studi microbiologici, interessandosi, in particolare di terreni di coltura per anaerobi 62. Col trasferimento, all’inizio del millennio, del museo di anatomia patologica nell’antico Istituto martinotti di via Irrerio, dove anche Tarozzi operò, sono stati ritorvati per puro caso alcuni preparati istologici autopsici della sua epoca. Forse non è un caso che questi preparati riguardassero patologia neoplastica, oggetto di accurate revisioni anatomicliniche da parte dei collaboratori del Tarozzi 63-64, le quali, come molti studi analoghi compiuti dai patologi bolognesi in altre epoche, hanno permesso di interpretare con maggior precisione diversi verbali dell’archivio autopsico di quel periodo.

Una serie di strisci senza coprioggetto ha costituito poi una sorpresa del tutto inattesa. Ripristinatili per la visione al microscopio, abbiamo visto che si trattava di bacilli del carbonchio e del tetano, fra l’altro, ancora in ottimo stato di conservazione. Si tratta sicuramente degli ultimi resti della collezione microbiologica di Giulio Tarozzi. Secondo il professor Mario Alberto Dina, oramai l’unico patologo vivente che conobbe Tarozzi, egli si occupò proprio anche di questi patogeni.

Dopo la guerra, Businco ricoprì l’insegnamento del’Anatomia Patologica presso diverse Università: nel’ordine, Perugia, Cagliari, Palermo, e di nuovo Cagliari, finché, nel 1938, non fu chiamato a Bologna, dove rimase fino al collocamento fuori ruolo, nel 1956. L’arrivo a Bologna corrispose con l’apice della fama nazionale del Businco. Egli si era in quegli anni particolarmente distinto per gli studi sull’endemia malarica in Sardegna. Egli teneva molto all’educazione dei medici sul problema della malaria, tanto da scrivere una monografia “L’infezione malarica” 68, che, col volumetto di tecnica delle autopsie, rivisitato negli anni novanta da Antonmaria Mancini 69, costituiva un supporto obbligatorio al testo di anatomia patologica sistematica per la preparazione dell’esame a Bologna. Prima e dopo la seconda guerra mondiale, Businco contribuì con grande passione alla lotta antimalarica in Italia. Oggi, questa grandiosa operazione è considerata con grande ammirazione dagli storici, tanto da essere suggerita come modello per i tanti paesi che ancora aspirano all’eradicazione di questo flagello. Dispiace che Businco, anche in opere recenti sull’argomento, non sia stato ricordato 70.

Al periodo bolognese è collegato un episodio drammatico e misterioso: nel 1944, Businco fu arrestato dai nazisti e deportato, ma riuscì a fuggire ed a nascondersi fino al termine della guerra. Prima della guerra, Businco non appariva in dissonanza col regime fascista. Ai tempi dell’occupazione tedesca, qualcosa doveva essere cambiato. Egli si era infatti opposto al tentativo degli occupanti di appropriarsi delle apparecchiature dell’Istituto del Radio; inoltre, era in contatto con un gruppo di studenti e giovani medici partigiani, molti degli altri istituti erano invitati a presenziare alle autopsie.

L’arrivo a Bologna corrispose con l’apice della fama nazionale del Businco. Egli si era in quegli anni particolarmente distinto per gli studi sull’endemia malarica in Sardegna. Egli teneva molto all’educazione dei medici sul problema della malaria, tanto da scrivere una monografia “L’infezione malarica” 68, che, col volumetto di tecnica delle autopsie, rivisitato negli anni novanta da Antonmaria Mancini 69, costituiva un supporto obbligatorio al testo di anatomia patologica sistematica per la preparazione dell’esame a Bologna. Prima e dopo la seconda guerra mondiale, Businco contribuì con grande passione alla lotta antimalarica in Italia. Oggi, questa grandiosa operazione è considerata con grande ammirazione dagli storici, tanto da essere suggerita come modello per i tanti paesi che ancora aspirano all’eradicazione di questo flagello. Dispiace che Businco, anche in opere recenti sull’argomento, non sia stato ricordato 70.

Dopo tanti anni, evidentemente, le acque non si sono ancora placate a sufficienza per spiegare con chiarezza che cosa fosse successo ad Armando Businco. Probabilmente, una spiegazione di tutto questo si trova nel dibattito ambiguo che la sanità bolognese venne a giocare fra il 1943 ed il 1945. L’Istituto Ortopedico Rizzoli e l’Istituto Traumatologico, diretti dal Professor Oscar Scaglletti, costituivano il più importante ed efficiente ospedale militare delle retrovie per le forze armate tedesche. Gli ufficiali italiani della croce rossa erano sicuramente in contatto con la Resistenza e con gli Alleati, sicuramente con una sorta di tacito consenso da parte dei Tedeschi. La tragedia di Businco e degli studenti di medicina fu forse “un’anomalia”, come asserisce il linguaggio diplomatico. Ciò spiega come Businco, pur essendo stato nominato presidente del comitato per l’epurazione, dopo la liberazione, di fatto, non riuscì ad andare oltre un ruolo puramente simbolico. Inoltre, gli strascichi giudiziari che ebbe il supposto collaborazionismo dei medici di Bologna, si chiusero con un “non luogo a procedere” (conservato nel gigantesco archivio di Armando Bu)

Businco era un deciso assertore della pratica delle autopsie 71, e riteneva che i clinici dovessero assistere personalmente al riscontro, per poter avere sempre un franco e non sterile scambio di idee col patologo. Gli stessi direttori degli altri Istituti erano invitati a presenziare alle autopsie. La completa ricostruzione dell’archivio autopicco universitario, dal 1838 al 1999, ha presentato qualche incongruenza col mito creatosi fra gli allievi del grande patologo sardo. Sino agli anni 20 del Novecento, le autopsie universitarie erano quasi puramente didattiche. Se
ne eseguivano soltanto da ottobre a giugno, e, all’epoca del Taruffi, i verbali autopsici, in fogli protocollo, erano scritti e firmati dagli studenti, con revisione del Taruffi e dei collaboratori. Ciò costituiva un preliminare d’ammissione all’esame vero e proprio. Abbiamo documentato il fatto che, conformemente alla tradizione di Malpighi e di Morgagni, i clinici del passato eseguivano personalmente l’autopsia sui propri malati, realizzando in tal modo quella che Juan Rosai vorrebbe rivitalizzare come “posthumous analysis”. Dopo la nascita dell’istopatologia moderna, il patologo era coinvolto dal clinico soltanto quando era necessario compiere uno studio istopatologico, a causa della necessità di conoscenze specifiche non alla portata di tutti i medici, e, soprattutto, dei costi d’un laboratorio istologico. Come abbiamo più volte sottolineato, fu la decisione di Virchow d’attribuire la gestione delle autopsie ai patologi, la causa del grande aumento dei riscontri autopsici nel Novecento, che a Bologna si manifestò negli anni venti, nell’ultimo periodo della direzione di Martinotti. Dopo, si ebbe una stabilizzazione, fino agli anni sessanta, quando iniziò un quasi inarrestabile declino, pur con pregevolissime eccezioni. Il dato veramente sconcertante è che l’introduzione della “dittatura” virchowiana introdusse un incredibile scadimento della qualità dei verbali autopsici, con una lenta, progressiva crescita di verbali inesatti. Ancora più sorprendente è lo scarso uso dell’istopatologia per la diagnosi, spesso con risultati perniciosi, come nella diagnosi di broncopolmonite. Questa situazione deludente, in un settore che è sempre stato considerato così vitale per i patologi, fu causa di ripetute prese di posizione da parte dei professori di anatomia patologica, a cominciare dal Martinotti, le quali, curiosamente, fanno riferimento ad una supposta ‘età dell’oro’ dell’autopsia, forse mai esistita, con l’eccezione dell’esclusiva scuola viennese e delle università da essa influenzate.

Crisi dell’autopsia a parte, già prima della guerra, contemporaneamente agli americani e forse indipendentemente da essi, data la barriera culturale che allora ce ne separava, il Businco aveva compreso i grandi cambiamenti cui andava incontro l’anatomia patologica, il miglioramento delle tecniche chirurgiche consentiva infatti un numero d’interventi sempre più elevato ed un crescente ricorso alla biopsia su organi prima inaccessibili. Ciò comportava per il patologo una diagnosi istopatologica di tipo nuovo e più difficile, la quale richiede una precisa conoscenza delle notizie cliniche e quindi uno stretto rapporto col clinico. Questo profondo travaglio dell’anatomia patologica moderna era particolarmente sentito dal Businco, e gli ispirò l’idea della necessità di un nuovo approccio, da lui denominato “approccio anatomoclinico all’anatomia patologica”, che già aveva discusso nella sua prolusione del 1938. Il suo insistere su ciò fu fondamentale per il rilancio dell’Istituto nei dopoguerra, quando le Scuole anatomopatologiche europee furono pervase dal nuovo concetto americano della Surgical Pathology.

In Businco troviamo i pregi ed i difetti dell’anatomia patologica, alla radice, probabilmente, della crisi d’identità che da tempo affligge questa disciplina. Dalla seconda metà dell’Ottocento sino agli anni Cinquanta, il patologo deteneva saldamente il controllo dei laboratori di medicina, esercitando in tal modo un’influenza sui clinici non proprio ben accetta. La rivendicazione di questo ruolo egemone è ben evidente in molti scritti del Businco, specialmente tra le due guerre mondiali. Egli auspica l’affidamento al patologo della direzione e del coordinamento di ogni grande clinica.

Come molti altri patologi, Businco percepì subito la grande importanza della radiologia, e la sua potenziale pericolosità per il controllo sulla medicina esercitato dal patologo. Proprio per questo egli riteneva che, trattandosi di scienze morfologiche, la radiodiagnostica dovesse appartenere all’anatomia patologica. Analogamente, auspicava il controllo della nascente radioterapia da parte dei patologi, a causa dell’impegno da essi profuso nella diagnosi oncologica.

Businco ebbe la fortuna di non vivere la crisi dell’anatomia patologica odierna. Riteniamo tuttavia che, forse, se fosse vissuto più a lungo, avrebbe compreso i nuovi orientamenti necessari alla rinascita della disciplina. Secondo noi, era un uomo di grande intuito, anche negli ultimi anni della sua lunga carriera. Proprio all’inizio del periodo bolognese, si accorse infatti del dilagare di una malattia poco frequente e, fino a quel momento, proprio per la sua relativa rarità, ancora mal conosciuta: il cancro polmonare. Negli ultimi anni della sua carriera, Businco s’interessò di altre patologie ‘alla moda’, come l’aterosclerosis e le malattie del sistema degli istiociti. Il cancro polmonare appare tuttavia per lui una fonte di autentica preoccupazione. In un decennio di pubblicazioni egli evidenzia il drammatico incremento del numero di queste neoplasie, e, negli anni Cinquanta, cerca di creare una classificazione razionale, basata, come sempre, su rilievi anatomico-clinici molto accurati. Da tutto questo deriva una pubblicazione molto articolata, poi tradotta in varie lingue, con accurati rilievi macroscopici ed istopatologici. Riteniamo che, se si eccettua la scoperta del microcitoma da parte di Azzopardi, ben poco sia stato aggiunto ai rilievi del Businco, sino allo sviluppo della patologia molecolare.

Un uomo così sensibile ai momenti di crisi della medicina sarebbe forse stato capace di guidare i patologi italiani ad una necessaria autocritica, attuando, nella teoria e nell’insegnamento dell’anatomia patologica, quelle modificazioni già verificatesi nell’attività professionale: la necessità di trasformare l’anatomia patologica in una materia che, come tutta la medicina di laboratorio, non è più il primo motore della medicina, ma una disciplina fedelmente al servizio della clinica.
Bibliografia


Cervical cancer screening programs in low-income communities. Experiences from Ecuador. Low cost detection of HPV infection in a developing country

G. CECCINI, G. PAGANINI, M. D’AMICO*, M. CANNONE*, C. BERTULETTI, M.C.P. BARBERIS**

Amici Fundation Terra Nueva Onlus, Ferrara; * Pathology Unit, Multimedica Group, Milan; ** Department of Pathology, Ospedali Riuniti di Bergamo, Italy

Key words

Ecuador • HPV screening • Archive smear • PCR

Summary

Objectives. With the support of the independent humanitarian organization “Amici Fundation Terra Nueva” in Quito, Ecuador, we evaluated the feasibility of a cytologic screening program sustained by volunteers on the field and in Italy.

Methods. 250 women underwent a cervical Pap-test. The women with a positive Pap-smear were re-called for visual inspection with acetic acid (V ia), whereas those with a negative smear were invited for a new Pap-test after 3 years. To obtain samples for molecular assays, cytologic material was removed from slides, submitted to DNA extraction and amplified by nested PCR of the L1 region of HPV DNA. PCR-positive samples were sequenced.

Results. Six (2.6%) samples showed squamous intra-epithelial lesions (SIls): 4 low grade and 2 high grade SIls were present in women more than 40 years old. The overall rate of successful DNA recovery on a per-slide basis was 96.5%. High grade SIls were characterized by HPV 16 and 18 co-infection. HPV 16 was detected in one low grade SIl. HPV-DNA was detected in 11 smears (4.95%): in all 6 SIls and in 5 of the 216 negative smears.

Conclusion. Independent humanitarian organizations could play a role in supporting national screening programs offering skilled field professionals and technical support by scientists operating in their countries. Our molecular technique has the potential to provide important epidemiological information in many resource-poor areas of developing countries.

Cervical cancer is the second most common cancer among women worldwide, with about 500,000 cases diagnosed annually. It reflects the global health inequity: there are dramatic differences between countries with efficient public health assistance and screening programs compared to developing countries in Africa, Latin America and Caribbean area where cervical cancer is the most common cause of death for cancer in women.

Human papilloma viruses (HPVs) cause virtually all cervical cancers with HPV genotypes 16 and 18 responsible for approximately 70%. Since recent studies on bivalent (HPV 16, 18) and quadrivalent (HPV 6, 11, 16, 18) vaccines showed a significantly lower incidence of high grade cervical intraepithelial neoplasia (CIN) in young women who had not been previously infected and received the vaccine, it is necessary to determine the distribution of oncogenic HPVs in different populations and geographic areas in order to estimate the potential benefits of the implementation and diffusion of vaccines. Little is known about the molecular epidemiology of HPV in Ecuador. The most cited epidemiologic study about the HPV–related cancers in Latin America, was published by the International Agency for Research on Cancer and it did not contain data on Ecuador. However, in Ecuador women with lower socio-economic status and less education have few chances to obtain a Pap smear as in most instances they do not have the financial resources.

With the support of the humanitarian organization “Associazione Amici Fundation Terra Nueva” in Quito, Ecuador, we studied the prevalence of HPV infection and HPV genotypes in women living in two different areas of this country: the town of Tosagua (region of Manabi) and the suburbs of Quito. The goal of the study was to determine the feasibility of a cytological screening

Correspondence

Massimo C.P. Barberis, Department of Pathology, Ospedali Riuniti Bergamo, largo Barozzi 1, 24128 Bergamo, Italy - Tel. +39 035 269054 - Fax +39 035 266176 - E-mail: mbarberis@ospedaliriuniti.bergamo.it
program sustained by field volunteers and cytotechnologists, molecular biologists and pathologists operating in Italy, offering their cooperation free of charge. A ‘home-brew’ molecular technique was used in order to reduce the costs and obtain information from the same stained smear used for cytological evaluation.

Patients and methods

We studied 250 women seeking a cervical Pap-test and attending two private health centers sustained by the Italian independent “Associazione Amici Terra Nueva Onlus” connected with Terra Nueva Hospital in Quito. The first group of women was enrolled in Tosagua, a rural community in the region of Manabi, not far from the Pacific coast. The population consisted of women living in poor hygienic conditions, usually with at least three or four pregnancies in their life beginning from 14-16 years. The second group was women attending a mobile screening service operating in the outskirts of the south of Quito City, the poorest quarters of the capital. Here, extremely low socio-economical conditions are associated with sexual promiscuity.

One hundred and twenty nine women from Tosagua and 141 from the outskirts of Quito were included in the study. All 270 participants were consecutively enrolled from September 2005 to October 2005. Exfoliated cervical cells using an Ayre spatula were collected by two expert gynecologists (GC and GP), smeared and fixed with polyethylene glycol. All the smears were sent by post to the Department of Pathology of Multimedica Hospitals, Milan, Italy, at a cost of 50 Euros. They were routinely stained and read by trained cytologists, and classified according to the Bethesda system. All abnormal smears and 20% of the negatives were re-examined by pathologists at the Pathology Unit of Ospedali Riuniti, Bergamo, Italy. The women with a positive Pap-smear were re-called at the Terra Nueva Hospital for visual inspection with acetic acid (VIA) and further therapy, if necessary, whereas the women with a negative smear were invited to perform another Pap-test after 3 years. The patients sustained only the costs for travelling from Tosagua to Quito.

To obtain samples for molecular assays, we used a previously described technique. Briefly, slides were immersed in acetone overnight to remove cover slips and then rinsed in xylene for 30 minutes and twice in ethanol; they were then air dried and the cytologic material removed by gentle scraping with a disposable blade and placed in a tube containing 300 μg/mL proteinase K in digestion buffer (50mM Tris-HCl, pH 7.5; 10 mM EDTA in 0.5% SDS; 50 mM NaCl). The samples were incubated overnight at 56°C and the DNA extracted using the phenol/chloroform method followed by ethanol precipitation. The amount of DNA was quantified by spectrophotometric determination (GeneQuant II, Pharmacia, Uppsala, Sweden). DNA integrity and absence of tissue amplification inhibitors was evaluated by PCR for HLA-DQα, as previously reported. Nested PCR was performed for the L1 region (open reading frame) of HPV DNA. Consensus primers (MY09, MY11) were used for outer amplification (450 base pair fragment). After the first round of the reaction, 3 μl of the amplified product underwent a second round of PCR using the inner primers GP5+ and GP6+, which amplify a 150 base pair fragment. Ten μl of the final product were electrophoresed on 2% agarose gel stained with ethidium bromide. The DNA extracted from HPV positive cervical biopsies was used as a positive control. Primers sequences are reported below:

MY09 5’-CGT CCM ARR GGA WAC TGA TC-3’
MY11 5’-GCM CAG GGW CAT AAY AAT GG-3’
GP5+ 5’-TTT GTT ACT GTG GTA GAT ACT AC-3’
GP6+ 5’-GAA AAA TAA ACT GTA AATCAT ATT C-3’

Amplification controls and detection limits were chosen and verified according to the methods described by Puranen et al. Briefly, amplification included positive (CaSki cells) and negative (sterile water) controls. The detection limit of the amplified sequence using gel electrophoresis and ethidium bromide staining alone was 20 SiHa cells. This represented approximately 20 copies of HPV DNA (20 fg HPV16 DNA). PCR-positive samples were purified with Qiaquick PCR purification kit, according to the manufacturer’s instructions (QIAGEN, Valencia, CA, USA). DNA sequencing was performed using an automatic DNA sequencer (ABI PRISM 3100, Applied Biosystems, Foster City, CA, USA). Sequence homology was determined by BLAST and ClustalW programs. HPV types considered “high risk” (HR) included all those described by Munoz et al.: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.

Results

There were 20 inadequate cytological samples (11 from Tosagua and 9 from Quito). Therefore, the study evaluated 250 specimens (118 and 132, respectively). The age of the women from Tosagua ranged from 24 to 68 years (mean 43), whereas the mean age of the Quito series was 49 years (range 21-72). Six (2.4%) of the 250 samples showed squamous intraepithelial lesions in the Papanicolaou stained smear. Table I shows the results of the Pap smear in women at the two health centers. Four high grade squamous intraepithelial lesions (SIL) were present in women more than 40 years old. All the SILs were characterized by HPV 16 and 18 co-infection. The same genotypes were also present in the four low grade SILs that occurred in women older than forty years. The incidence of Pap smear alterations was the same in the two series. The overall rate of successful DNA recovery on a per-slide basis was 88.8% (222 of 250 specimens). HPV-
DNA was detected in 11 smears (4.95%): in all 6 squamous intra-epithelial lesions and in 5 of the 216 negative smears. The PCR results are reported in Table II.

### Discussion

Cancer of the uterine cervix is a major public health problem in Latin America. In most resource-poor settings, cytological screening has proven difficult to implement and sustain, in large part because this form of screening relies on highly trained cytotecnologists, high quality laboratories and a logistic organization to support screening, colposcopy and treatment. In Ecuador, additional issues are the jeopardization of resources and the exclusion of a number of citizens from public health facilities both in the metropolitan area of Quito and in rural areas simply because they cannot sustain costs.

Our study showed the feasibility of a small screening program proposed by a humanitarian organization operating for many years in Ecuador with limited resources, but sustained by volunteer professionals who devote a small part of their time. Only the direct health care costs are be covered by the organization (screening test, clinic visit, laboratory tests, specimen transport, subsequent visits for treatment). The direct non-health care costs such as child care costs for a mother in treatment and transport costs for treatment were covered by families.

In this study, we found an HPV infection rate of 4.95% in women seeking a Pap test in two different areas of Ecuador. This prevalence is somewhat lower than those reported in other countries in Latin America, such as Mexico (14%) \(^1\), Chile (14%) \(^2\), Colombia (16.8%) \(^2\), and Argentina (15.5%) \(^2\). The prevalence found in this study is quite similar to those observed in Bolivia (5.2%) \(^1\) and in the state of Durango, Mexico (4.8%) \(^2\). These two studies considered women living in rural vil-

<table>
<thead>
<tr>
<th>Cytological diagnoses</th>
<th>Quito</th>
<th>%</th>
<th>Tosagua</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSIL</td>
<td>2</td>
<td>1.5</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>HSIL</td>
<td>1</td>
<td>0.7</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>2.2</td>
<td>3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

### Table II. Results of PCR for stained cervical smears.

<table>
<thead>
<tr>
<th>Cytological diagnoses</th>
<th>HPV-DNA positive</th>
<th>HPV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>244</td>
<td>97.6</td>
</tr>
<tr>
<td>LSIL</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>HSIL</td>
<td>2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Low risk: 42, 66, 81
High risk: 16, 18
radiation of DNA caused by fixation and staining. This last point has been exhaustively discussed by Canfell et al. The high recovery rate of DNA that we obtained was due to the use of reagents and dyes without acetic acid in order to avoid hydrolysis of glycosidic bonds and subsequent generation of basic DNA sites, which may adversely impact Taq polymerase activity during the extension phase of the PCR. Similar results were obtained in a previous study in Netherlands, which reported a DNA recovery rate of 98%.

The technique is robust and inexpensive: DNA extraction and amplification is available at a direct cost of less than 10 Euros per sample, and HPV genotyping by sequence analysis has similar costs. Commercial kits for high and low risk HPV detection cost 5-10 times more. Independent humanitarian organizations can play a role in supporting national screening programs by offering skilled field professionals and technical support by scientists operating in their countries. The poorest part of population that are excluded from any form of screening can be reached at least in part by these organizations and obtain benefits in terms of early detection of cervical cancer. A continuous education project in cytology and molecular techniques for local technicians and biologists is pending economic support.

References


Liquid-based endometrial cytology: 
the Florence and Bari experience

A.M. BUCCOLIERO, L. RESTA*, A. NAPOLI*, G.L. TADDEI
Department of Human Pathology and Oncology, University of Florence, Italy; *Department of Pathology, University of Bari, Italy

Key words
Thin-layer • Liquid-based • Endometrial cytology • Endometrial adenocarcinoma • Endometrial hyperplasia

Summary
Several diagnostic procedures are available to investigate the endometrium, i.e. sonography, hysteroscopy, biopsy, endometrial curettage and cytology. Among these, endometrial cytology is less commonly utilized. Although the use of cytology in the diagnosis of endometrial adenocarcinoma has already been proposed due to its low cost and simple execution, a general consensus has not been reached. The improvement of the diagnostic capacity of endometrial cytology following the introduction of a liquid-based method suggests that this test should be routinely used in endometrial diagnosis. The main advantages of this method are the reduction in confounding factors, the distribution of cells on a thin layer and the possibility to obtain more slides from the same sample. The aim of this article is to focus on the methodological procedures and diagnostic criteria in liquid-based endometrial cytology based on the experience in two Italian centres: Department of Pathology, University of Bari and Department of Human Pathology and Oncology, University of Florence. The sampling method used by the Bari authors consists in the collection of liquid for uterine distension during hysteroscopy, while the Florence group used an endometrial brush. The sensitivity and specificity at Bari were 75% and 83%, respectively, and were 94-100% and 95-100% at Florence, respectively. Endometrial cytology provided sufficient diagnostic material significantly more often than biopsy. We thus propose that endometrial cytology can be used in routine diagnosis either alone or in association with other diagnostic procedures in order to improve diagnostic accuracy.

Introduction
Several diagnostic procedures are available to investigate the endometrium, i.e. sonography, hysteroscopy, biopsy, endometrial curettage and cytology. Among these, endometrial cytology is less commonly utilized. Although the use of cytology in the diagnosis of endometrial adenocarcinoma has already been proposed due to its low cost and simple execution, a general consensus has not been reached. The diffusion of conventional endometrial cytology has been hampered by difficulty in its interpretation due to the presence of excess blood, mucus and overlapping cells. Improvements in the diagnostic accuracy of endometrial cytology related to the introduction of the liquid-based methodology suggest that the test could be routinely used in endometrial diagnosis. The main advantages of the method are the reduction in confounding factors, the distribution of cells on a thin layer and the possibility to obtain more slides from the same sample. The possibility of cytological examination of the endometrium is particularly useful in post-menopausal women. Indeed, endometrial atrophy may determine a high inadequacy rate of endometrial biopsies. In this context, endometrial cytological samples have been shown to be diagnostic in a significantly higher percentage of cases with respect to biopsy samples (82% vs. 24%) 1. Moreover, thin layer endometrial cytology appears to fill a large diagnostic gap in endometrial pathology, namely recognition of a small cancer arising in atrophic mucosa and characterization of tamoxifen-induced changes that are not easily detectable with biopsy, hysteroscopy or sonography.

The use of the thin layer endometrial cytology is encouraged by its excellent diagnostic accuracy, particularly in cases of endometrial carcinoma and atypical hyperplasia, in which the method may be compared with the secular experience of endometrial biopsy 2-10.

The aim of this article is to focus the methodological procedures and diagnostic criteria in liquid-based endometrial cytology based on the experience in two Italian centres (Department of Pathology, University of Bari and Department of Human Pathology and Oncology, University of Florence).
Methodological procedures and patients

**BARI**

This method is proposed for women who undergo hysteroscopy using physiological solution for uterine corpus distension. As a cytological sample, the first 20 ml of liquid used in uterine distension is collected and promptly processed in the cytology laboratory as follows:

- centrifugation at 1,200 rpm/10 min;
- suspension of the pellet in 50 ml of Cytolyt\textsuperscript{®} (Hologic), saline solution with a low part of ethanol for lysis of erythrocytes, granulocytes and mucus;
- lysis for 20-30 min;
- centrifugation at 1,200 rpm/10 min;
- the pellet is suspended in PreservCyt\textsuperscript{®} (Hologic), a methanol-based solution used as a bactericide and fixative;
- preparation of slides with “Thin-prep 2000” (Hologic);
- post-fixation with 95% ethanol /30 min;
- Papanicolaou staining;
- observation by standard light microscopy.

This methodology was used preliminarily in 97 patients with a history of abnormal uterine bleeding, often in menopause, under tamoxifen treatment or infertility.

**FLORENCE TECHNIQUE**

Cytological sampling is performed by endometrial brushing using the Endoflower device (RI-MOS). After endometrial sampling, the device tip is immersed in the Cytolyt\textsuperscript{®} vial where it is vigorously rotated to facilitate cell release, the sample is sent to the cytology laboratory where it is processed as follows:

- centrifugation at 1,200 rpm/10 min;
- removal of the supernatant
- suspension of the pellet in 50 ml of Cytolyt\textsuperscript{®} for erythro- mucolysis;
- lysis in 20-30 min;
- centrifugation at 1,200 rpm/10 min;
- removal of the supernatant;
- (in case of persistence of blood and mucus, additional centrifugation, supernatant removal and suspension in Cytolyt\textsuperscript{®} may be performed)
- the pellet is suspended in PreservCyt;
- preparation of slides with “Thin-prep 2000”;
- post-fixation with 95% ethanol/30 min;
- Papanicolaou staining;
- observation by standard light microscopy.

Since 2001, liquid-based endometrial cytology has been routinely used at the Department of Human Pathology and Oncology of Florence Italy. During this period, we assessed the diagnostic accuracy of the liquid-based method in endometrial diagnosis through several cyto-histological concordance studies. We ascertained its effectiveness in different aspects of endometrial pathology: asymptomatic women (320 patients)\textsuperscript{10}, thickened endometrium as evaluated by trans-vaginal sonography (670 patients)\textsuperscript{5}, and women on tamoxifen (168 patients)\textsuperscript{4}.

Diagnostic criteria

**STATEMENT ON SPECIMEN ADEQUACY**

Specimens are considered inadequate for diagnostic evaluation when they contain less than 6 epithelial endometrial cell clusters. Moreover, specimens are considered unsatisfactory for diagnosis when insufficient clinical information (i.e. age, menopausal state, menstrual state, hormonal therapy, risk factors, symptoms) is provided.

**DIAGNOSTIC CRITERIA**

Diagnostic criteria are based on cyto-architectural evaluations and consider the epithelial and stromal (Fig. 1) endometrial cells as well as the background\textsuperscript{11}. *Proliferative endometrium* (Fig. 2) is characterized by the presence of three-dimensional cylindrical epithelial endometrial clusters. Cytoplasm is scant. Nuclei are isomorphic with finely granular chromatin. Nucleoli are small or absent. Cellular polarity is preserved. Stromal...
cells are abundant and spindle-shaped. The background is clean. *Secretory endometrium* (Fig. 3) shows wide, three-dimensional cylindrical epithelial clusters. Bi-dimensional placards may be present in the late secretory phase. Cytoplasm is clear and obvious. Nuclei are isomorphic with dispersed chromatin and small or absent nucleoli. Cellular polarity is preserved. Stromal cells are abundant and decidualized (wide cytoplasm, round nuclei, finely granulated chromatin, micro-nucleoli). The background is clean or in the late secretory phase is moderately inflammatory. *Endometrial atrophy* (Fig. 4) is characterized by the presence of small cylindrical three-dimensional epithelial clusters. Epithelial clusters may appear swollen in cystic atrophy. Cytoplasm is scant. Nuclei are isomorphic with dense chromatin and small or absent nucleoli. Cellular polarity is preserved. Stromal cells are abundant and spindle-shaped. The background is clean. Multinucleated histiocytes are often recognizable. *Hormonal administration* (Fig. 5)

**Fig. 3.** Secretory endometrium: wide, three-dimensional cylindrical epithelial cluster (clear and obvious cytoplasm, isomorphic nuclei, preserved cellular polarity). (PAP-stain; original magnification 5X).

**Fig. 4.** Atrophic endometrium: small cylindrical three-dimensional epithelial cluster (scant cytoplasm, isomorphic nuclei, dense chromatin, preserved cellular polarity) and multinucleated histiocyte. (PAP-stain; original magnification 10X).

**Fig. 5.** Progestin effects: secretory endometrial gland and decidualized stroma (wide cytoplasm, round-oval nuclei, finely granulated chromatin, micro-nucleoli). (PAP-stain; original magnification 20X).

**Fig. 6a-6b.** Endometrial hyperplasia: wide three-dimensional epithelial endometrial clusters with cellular crowding and architectural disorder. (PAP-stain; original magnification: 6a, 20X; 6b, 10X).
leads to endometrial morphological modifications that depend on the type of hormone administrated, dosage, regimen (combined or sequential estro-progestin administration) and duration and in fertile women the menstrual phase in which the hormone is administrated. Oestrogens, when unopposed by progestins, produce a proliferative input on the endometrium determining possible hyperplastic and even neoplastic progression. In contrast, progestins are responsible for proliferative arrest, glandular secretion and decidualization of stromal cells. Prolonged progesterone treatment induces progressive arrest of secretions and glandular atrophy. Cytological features in endometrial specimens reflect these hormonal induced modifications. Endometrial Hyperplasia (Figs. 6a-6b) appears in cytological samples as numerous, wide, three-dimensional epithelial endometrial clusters with variable cellular crowding and architectural disorder. In typical endometrial hyperplasia, the cytoplasm is commonly scant and the nuclei are isomorphic with finely granular chromatin and small or absent nucleoli. In atypical endometrial hyperplasia, the cytoplasm becomes evident and nuclei may show a moderate-grade of pleomorphism. Spindle-shaped stromal cells are abundant in typical hyperplasia, while they are less represented in atypical hyperplasia. The background may enclose inflammatory cells. The main diagnostic criteria for endometrial carcinoma (Figs. 7-10) are: 1. architectural (loss of polarity, papillary cell clusters, dyshesive cells); 2. cellular (high nucleo/cytoplasmatic ratio, anisonucleosis, coarse and/or marginalized chromatin, nucleolar prominence, nuclear membrane incisures, cell cannibalism); 3. background (scarcity of stromal cells, inflammation, necrosis). In the case of well differentiated (G1) endometrioid adenocarcinoma, epithelial endometrial clusters are medium in size and show cellular overlapping. Cellular polarity is lost, at least in part. Cytological atypia is unremarkable and tumour diathesis is not a constant feature. In case of poorly differentiated (G3) endometrioid adenocarcinoma, epithelial endometrial clusters are small and less crowded with respect to endometrial hyperplasia and well differentiated adenocarcinoma. Cytological atypia is prominent. Cell cannibalism is observed more often in poorly differentiated tumours. Specimens obtained from patients affected by serous carcinomas are hypercellular (small cellular clusters with inconspicuous cellular...
crowding, single cells, bare nuclei). Psammoma bodies can sometimes be seen.

Results

Bari results

The number of inadequate specimens was 6/29 in the group of endometrial cancer; 6/20 in the group of tamoxifen-treated and 3/48 in patients with infertility, abnormal uterine bleeding or endometritis. The correlation between cytology and histology (when performed) revealed a sensibility of 75% and a specificity of 83%. Most inadequate cases and false positive or negative samples were seen in the initial experience before the technique was improved upon (Tab. I).

Florence results

For all samples, endometrial cytology provided sufficient material more often than biopsy (P < 0.01).

The sensitivity and specificity were estimated, respectively, at 94-100% and 95-100%; the positive and negative predictive values were estimated, respectively, at 80-100% and 99-100% (100% values were obtained in 33 women on tamoxifen in which both endometrial cytology and biopsy gave adequate specimens).

Conclusions

The improvement of the diagnostic capacity of endometrial cytology by introduction of a liquid-based method should persuade both clinicians and pathologists to definitively introduce this test in routine endometrial diagnosis. Indeed, the characteristics of the thin layer method, namely reduction of confounding factors, distribution of cells in a thin layer, and the possibility to obtain more than one slide that can be used for further investigation, i.e. immunohistochemistry and molecular biology, are all useful for endometrial diagnosis. The low percentage of unsatisfactory specimens, and the high sensibility, specificity, and positive and negative predictive values from both the Bari and Florence experience, as well as in a number of recently published studies, support this belief.

In conclusion, we consider endometrial cytology an efficacious diagnostic procedure. It can be applied either alone or in association with other diagnostic procedures to improve diagnostic accuracy.

References

Primary linitis plastica of the right colon

M. ONORATI, M.R. AMBROSIO, V. MOURMOURAS, M.G. MASTROIULIO, B.J. ROCCA
Department of Human Pathology and Oncology, Section of Anatomic Pathology, University of Siena, Italy

Key words
Linitis plastica • Right colon

Summary
A case of primary linitis plastica of the colon is presented. This case is of interest for three reasons: the site of origin in the right colon (80% of cases reported develop distally to the splenic flexure), a biopsy previously taken from the mucosa demonstrated the presence of a signet ring cell carcinoma (endoscopic biopsies do not provide a conclusive diagnosis in the majority of cases reported) and hyaline with sparse amyloid nodules were detected in the extensive, dense fibrous tissue intermingled with tumour cells.

Introduction
The term “linitis plastica” was introduced by Lietaud in 1779, followed by Andran in 1829 and Brinton in 1859, and is considered as a gastric inflammatory-like neoplastic lesion (sicut retis ex lino facta) that produces abundant fibrous tissue (plastica = desmoplastic) in the stomach wall. It is interesting to note how Antonio Benivieni described this lesion in 1507 “stomachum fere totum obcaluisse repertum est”.
Primary linitis plastica of the colon was first described by David in 1931 and was defined by Lauerman and Saphir in 1951 as a neoplastic lesion characterized by a conspicuous, prevalently submucosal, desmoplastic reaction to various histotypes of infiltrating carcinoma. The colon wall is considerably thickened, but in many cases the mucosa has a normal appearance. Based on previous knowledge and a higher frequency of gastric linitis plastica, it is always necessary to exclude secondary colon involvement by a gastric carcinoma, which has been recognized as a possibility since its first description by Coe (1931). To our knowledge, only about 60 cases of linitis plastica of the colon have been described. Herein, we report a case originating in the right colon in the absence of gastric lesions.

Case report
A 69-year-old man was admitted to the Surgery Unit of Nottola Hospital (Siena) with severe abdominal pain. The only remarkable event in his medical history was a prostatic adenocarcinoma treated with radical surgery and radiotherapy. The abdomen was tense and painful, with lower right dullness and hyperperistalsis. Haemoglobin was 12.4% and haematocrit 32.9%. Colonoscopy revealed a stricture involving the caecum and the proximal portion of the ascending colon; a biopsy revealed a signet ring cell carcinoma. Computerized tomography revealed a neoplasm of the caecum and ascending colon, located cranially to the ileo-cacal valve, and appeared mainly extrinsic. Lymph nodes were enlarged and there were peritoneal implants. Apart from the ileum no other abdominal or pelvic organs were involved. Gastroscopy and gastric biopsies were negative for neoplasia. At laparotomy, ascites and diffuse peritoneal and omental carcinomatous implants were seen, without liver metastases. The carcinoma extended transmurally through the wall of the caecum and terminal ileum, which appeared rigid and thickened. The adjacent soft tissues were hardened. A right hemicolectomy (12 cm) and a terminal ileum resection (30 cm) were performed. Pericolic and peri-ileo tissues and several lymph nodes were removed. The intestinal loops were conglomerated, rigid and thickened due to a mass encircling them and considerably reducing their lumen. However, only a part of the mucosa appeared to be involved, where it was eroded and haemorrhagic. Tissues taken from all involved areas were included in paraffin. Sections were stained with haematoxylin and eosin, Alcian blue and Congo red, while others were prepared for immunohistochemistry and stained for the following...
antibodies: cytokeratin AE1/AE3, 7 and 20, CDX2, chromogranin, synaptophysin and PSA. Histologically, at low magnification, the carcinoma was composed of nests and clusters of small, low cuboidal cells with basophilic cytoplasm and vesicular nuclei that sometimes formed abortive glandular structures (Fig. 1a). Some of these glands showed a mucinous content in their small luminal spaces and the ability to produce an intracytoplasmic mucinous secretion. Clusters of neoplastic cells were distorted and dislocated by dense collagenous tissue that was occasionally structured in concentric bands to form discrete hyalinized nodules (Fig. 1b). At higher magnification, nests of signet ring cells were visible in the lower part of the mucosa (Fig. 1c, d). In the sections stained with Alcian blue, isolated or small clusters of signet ring cells were intermingled with small, cuboidal cells (Fig. 2a) and scattered throughout the wall and in the stromal tissues. Under polarized light, a moderate green birefringence was visible inside these nodules in the sections stained with Congo red (Fig. 2b). Both the cuboidal cells and the signet ring cells were positive for CDX2 (Fig. 2c), CK AE1/AE3. They were also positive for CK20 (although not diffusely) and for CK7 (only focally), while they were negative for chromogranin and synaptophysin.

Infiltration by prostatic adenocarcinoma was excluded based on the morphology and negativity for PSA. Vascular and perineural invasion was detected. Lymph node metastases were observed. The diagnosis was “poorly differentiated adenocarcinoma with extracellular mucinous and signet ring cell carcinoma components, pT4N2Mx C2/III G3”.

**Discussion**

There are several reasons for describing this case of linitis plastica of the colon. To date, only about 60 cases have been reported in the literature. Our case originated in the right colon, which is unusual as 80% of primary linitis plastica of the colon develops distally to the splenic flexure. An endoscopic biopsy taken from an area of eroded and haemorrhagic mucosa demonstrated the presence of a signet ring cell carcinoma. This was also unusual since the mucosa was mainly spared in the majority of cases reported, meaning that endoscopical-
Primary linitis plastica of the right colon

ly directed biopsies not including the submucosa can rarely provide a conclusive diagnosis. The tumour histotype consisted of two cell populations. The first was small, low cuboidal cells with basophilic cytoplasm and vesicular nuclei, which formed abortive glands with luminal and cytoplasmatic mucin content or were aggregated in cords and clusters. The second population consisted of nests of signet ring cells in the deep part of the mucosa, which were easily visible with Alcian blue, CDX2, CK20 and CK7 and were scattered throughout the colon wall, even inside the aggregates of cuboidal cells. The positivity of both these components for CDX2 and for CK20 (although not diffusely) favours a large bowel origin of the tumour and constitutes a histogenic link between the two populations of neoplastic cells. As in the majority of cases reported, extensive dense fibrous tissue intermingled with clusters of tumour cells was evident and especially notable within the submucosa and muscularis propria. Discrete hyalinized nodules were present in the fibrous tissue, and a moderate green birefringence was visible within them under polarized light in the sections stained with Congo red.

Similarly to previous reports, the tumour showed aggressiveness since it invaded vessels in the colon wall as well as soft tissues, the ileum and lymph nodes, although evident visceral metastases were absent.

References


6 Amorn Y, Knight WA. *Primary linitis plastica: report of two cases and review of literature*. Cancer 1978;41:2420-5.

9 Balthazar EJ, Rosenberg HD, Davidan MM. Primary and metastatic scirrhous carcinoma of the rectum. AJR 1979;132:711-5.
11 Bonello JC, Quan SHQ, Sternberg SS. Primary linitis plastica of rectum. Dis Colon Rectum 1980;23:337-42.
CASE REPORT

Renal hilus paraganglioma: a case report and brief review

F. PAGNI, E. GALBIATI, F. BONO, C. DI BELLA
University Milan “Bicocca”, Desio Hospital, Italy; University Milan “Bicocca”, Nephrology Department, Monza San Gerardo Hospital, Italy

Key words
Paraganglioma • Renal hilus • Extraadrenal paraganglioma • Extraadrenal pheochromocytoma

Summary
Paraganglioma is a rare tumour that originates from any paraganglia. Among extra-adrenal paraganglioma, renal hilus is a rare location. The authors report a case of a 43-year-old female who was admitted for evaluation of a renal mass detected incidentally by ultrasound imaging. Suspecting malignancy, the patient underwent radical nephrectomy. Upon macroscopic examination, the lesion was located into the renal hilus. Histological study revealed a neoplasm constituted of nests of monomorphic cuboidal cells with basophilic granular cytoplasm and round to oval nuclei. Necrosis was absent. The proliferative index (Mib-1) was very low (<5%). Immunohistochemical examination revealed reactivity for neuron specific enolase (NSE), chromogranin A and synaptophysin. The final diagnosis was renal hilus paraganglioma. The paper shows the difficulty in diagnostic approaches to paraganglioma in this atypical site.

Case report
A 43-year-old woman was submitted to renal ultrasoundography for a 1 month history of abdominal pain. The patient was normotensive; all laboratory data, including endocrinologic tests, were within normal ranges and there were no pathologic signs in conventional urine tests. During an ultrasound exam, a mass was detected close to the right kidney. Abdominal and pelvic CT showed no abnormalities in the bilateral adrenal glands, but a large, partially cystic and encapsulated mass (9 cm in maximum diameter) was localized near the renal hilus. There were no nodal or distant metastases. The initial radiological differential diagnosis included renal cell carcinoma and pelvis transitional cell carcinoma. Based on this, the patient underwent right radical nephrectomy. Frozen sections were not performed. By macroscopic examination, the kidney showed a red to brownish mass, solid and cystic, partially haemorrhagic, with a central white scar, located in the renal hilus and detachable from the contiguous renal tissue (Fig. 1). Histology revealed a neoplastic mass arising from the kidney, in the adipose tissue of the hilus (Fig. 2), which consisted of nests of cuboidal, monomorphic cells with basophilic granular cytoplasm and round to oval nuclei without severe nuclear atypia (Fig. 3). A prominent fibro-vascular stroma was present. Neoplastic cells were positive for neuron specific enolase (Fig. 4a), chromogranin A (Fig. 4b) and synaptophysin (Fig. 4c), and were negative for AE1-AE3 cytokeratins (Fig. 4d). Only a small number of S-100 protein positive spindle shaped cells (sustentacular cells) were detectable around the tumour nests. Mitotic figures were virtually absent; proliferative index, evaluated by Mib-1, was <5%.

Correspondence
Dr Fabio Pagni, University Milan Bicocca, Pathology Department, via Cantore 8, 20052 Monza, Italy - E-mail: petala.83@tiscali.it
Fig. 2. The neoplasm (lower left) was separated from the normal kidney (upper right) and was situated in the adipose tissue of the renal hilus (H&E;1x).

Fig. 3. "Zellballen" characteristic pattern of growth with classic fibrovascular stroma of paraganglioma (haematoxylin and eosin; 20x).

Fig. 4. Immunohistochemical staining for enolase neurone specific (Fig. 4a), chromogranin A (Fig. 4b), synaptophysin (Fig. 4c); no immunoreactivity for keratin AE1/AE3 was observed (Fig. 4d).
There was no evidence of capsular or vascular invasion. Accordingly, a final diagnosis of paraganglioma of the renal hilus was made.

**Materials and methods**

Immunohistochemical analysis was performed using the following antibodies: S-100 protein (polyclonal, Dako), NSE (BBS/NC, Dako), synaptophysin (polyclonal, Zymed), chromogranin A (polyclonal, Zymed), Ki-67 (Mib-1, monoclonal, Dako), cytokeratin (AE1-AE3 monoclonal, Dako).

**Discussion**

Regardless of location, paraganglioma is defined as a tumour arising from any paraganglia. Historically paraganglioma that arise from adrenal medulla is defined as “pheochromocytoma”, and extra-adrenal paragangliomas that are associated with clinical evidence of epinephrine secretion are defined as “extra-adrenal pheochromocytomas”. However, there are no histological criteria to distinguish between functioning or non-functioning tumours. The functioning form of this tumour can lead to clinical manifestations: in this case, diagnosis may be easier but prognosis is more difficult since surgical manipulation of the neoplastic mass can produce a hypertensive crisis. Paraganglioma may develop in the context of type IIA and IIB MEN syndrome, neurofibromatosis, von Hippel-Lindau syndrome or Carney syndrome, but most frequently it arises spontaneously. The most common site is the carotid body at the bifurcation of the common carotid artery. In other cases, the tumour arises from the retroperitoneal paravertebral chain, which is why the term Zuckerkandl’s body paraganglioma is used.

This entity is usually found next to the angle formed by the aorta and the origin of the inferior mesenteric artery. A search of the literature revealed 5 reported cases of renal paraganglioma and 6 reported cases of renal pheochromocytoma up to 2005. In 2007, Yamamoto described a new malignant case arising in the kidney of a 34-year-old man. Table I shows the previous 12 case reports and details the characteristic clinical findings. The patient-age ranged from 27 to 68 years old with a clear predominance in males. Interesting variants included cystic paragangliomas, giant cystic paraganglioma and malignant pheochromocytoma. Relevant clinical findings included association with carotid paraganglioma, appendiceal mucocele and renal pheochromocytoma with renal artery stenosis. In one case, in a 27-year-old man, only cDNA microarray analysis assisted in diagnosis of malignant intrarenal pheochromocytoma that was originally masquerading as a renal cell carcinoma. Because of its rarity, especially in non-functioning cases, paraganglioma can create problems in preoperative differential diagnosis.

In our case, radiological examination demonstrated the connection of the tumour with the kidney, but it was not possible to better define the lesion. In fact, the absence of clinical secretions and the anatomical independence of the mass from the adrenal gland persuaded the surgeons to surmise a renal malignant tumour. Therefore, only histology could define the lesion. Histological differential diagnosis included many entities. First of all, it was important to rule out the possibility of a neuroendocrine carcinomas: keratins were negative as in all extra-adrenal paragangliomas, with exception of those arisen from filum terminale. We next excluded a primitive neuroectodermal tumour (PNET) of the kidney based on morphological and phenotypic criteria. This is a rare and highly aggressive malignancy that can also present with inferior vena caual thrombus. Immunohistochemistry

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<td>Ann Urol (Paris)</td>
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<td>Takahashi, 2005</td>
<td>J Med Genet</td>
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<td>Guate Ortiz, 2000</td>
<td>Arch Esp Urol</td>
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<td>Rossi, 2001</td>
<td>J Urol</td>
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<tr>
<td>Melegh, 2002</td>
<td>Pathol Res Pract</td>
<td>Giant cystic variant</td>
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<td>Rafique, 2003</td>
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<td>Eur J Surg Oncol</td>
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<td>Yamamoto, 2007</td>
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revealed strong positivity for CD99 and weak positivity for vimentin. Neuron-specific enolase (NSE), chromogranin and cytokeratin were negative. Finally, we considered the possibility of a schwannoma of the kidney. This is a rare condition with only a few reported cases. Schwannoma is totally or partially encapsulated; it is diffusely positive for S100 protein and neuron-specific enolase. Immunostaining for neurofilament, HMB45, microphthalmia transcription factor, smooth muscle actin, CD34, cytokeratin AE1/3, cytokeratin 7, and CD10 were negative.

In adrenal paraganglioma, the literature proposes the “rule of 10s” because about 10% of paragangliomas has familiar incidence, 10% present as multiple tumours, 10% have childhood onset, 10% recur after surgery and 10% are malignant. Although extra-adrenal paragangliomas are very similar to adrenal ones, malignant paragangliomas in extra-adrenal sites are more frequent: 29-40% versus 2-11%. According to the literature, the clinical and histological features predictors of malignancy are male sex, size over 5 cm, high mitotic activity, presence of confluent necrosis, vascular or capsular invasion, secreting tumours and decreased immunoreactivity for neuropeptides. On the contrary a high representation of S-100 protein positive sustentacular cells in the histological specimen should be associated with a better clinical course. Nonetheless, differentiation between malignant and benign cases may be very difficult and only the clinical behaviour (such as the evidence of metastases) could be a definitive criterion. As in our case, the treatment of choice and the best predictor of no recurrence is the surgical complete removal of the mass. In fact, in the absence of guidelines for management of these neoplasms, patients undergo clinical and ultrasound examination every 6 months for the first 5 years after surgical treatment.

References
Endometrial stromal sarcoma presenting as a cystic abdominal mass

Pathology Department of Salah Azaiez Institute, Bab Saadoun, Tunis, Tunisia

Summary
Endometrial stromal sarcoma (ESS) is rarely localized in extra-uterine sites if metastasis or local extension of the primary uterine tumour are excluded, and diagnosis can be delayed because of the unusual site. We report a case of abdominal ESS in a 45-year-old woman who presented with an abdominal complaint. Ultrasound of the abdomen showed a large multiloculated cystic mass. The complete excision of the tumour revealed ESS arising in endometriosis. The tumour expressed hormonal receptors and the patient was administered hormonal therapy. ESS has a better prognosis than the sarcoma that is part of differential diagnosis, and is associated with endometriosis in about one-half of cases.

Introduction
Endometrial stromal sarcoma (ESS) is an uncommon neoplasm of low grade of malignancy according to the WHO classification. The occurrence of ESS outside the uterus is extremely rare in the absence of metastasis or extension of a primary uterine neoplasm; it is then easily misdiagnosed in extra-uterine sites. When ESS occurs in an extra-uterine location, it presumably arises from foci of endometriosis, which should be carefully investigated.

Case report
A 45-year-old woman, gravida 2/para 2, presented with complaints of abdominal discomfort lasting 6 months. She has no significant medical history. Physical examination revealed a large palpable abdominopelvic mass. Ultrasound showed a large multiloculated cystic mass measuring 29 cm in maximum dimension. The inner lining of cysts revealed multiple papillary projections measuring up to 0.1 cm in the greatest dimension. Uterus and adnexa were within normal size. Surgical investigation revealed an enlarged tumour measuring 35 cm in diameter. The abdominal cystic mass was reported as completely excised and appeared to have no involvement with any intra-abdominal organs except the omentum.

Gross findings
The surgical specimen included the abdominal mass and the omentectomy. The mass measured 35x28x18 cm. The external surface was smooth. Sections showed a large multilocular cyst filled with haemorrhagic fluid that occupied the major volume of the tumour. The inner lining of cysts was orange-yellow or tan-grey to slightly yellow (Fig. 1). The solid areas were firm, with a whorled cut surface and focal haemorrhage. Another specimen submitted as “epiploon surrounding abdominal mass” measuring 22x20x2 cm. It consisted of fatty tissue with no suspect nodules.

Microscopic findings
The abdominal mass had hypercellular zones mixed with areas of lower cellularity, irregular infiltrating borders and several foci of necrosis. The tumour was arranged in a diffuse and slightly whorled pattern. Cells were oval to spindle-shaped with a scant cytoplasm (Fig. 2). Cell nuclei were of uniform size with smooth contours, distributed chromatin and indistinct nucleoli. Mitotic figures were extremely rare and when found were normal in appearance (3 mitosis/10 HPF). No polymorphic or enlarged nuclei were found. Some bland glands with a single epithelium were intimately admixed with tumoural cells (Fig. 3). The large cystic...
walls were lined by a discontinuous endometrial epithelium. A prominent plexiform vasculature was noted. In peripheral areas, extensive stromal hyalinization, sheets of foamy cells and cholesterol deposits were present.

Immunohistochemical studies showed that the neoplastic cells were immunoreactive to CD10 (Novocastra®) (Fig. 4a), and both oestrogen receptor and progesterone receptor antibodies (Novocastra®) showing diffuse strong nuclear staining (Fig. 4b). Actin (Dako®) was weakly positive, desmin (Dako®) and inhibin (Zymed®) were negative.

All these features were compatible with a diagnosis of low grade endometrial stromal sarcoma arising from pelvic endometriosis.

Discussion

Low grade endometrial stromal sarcoma is an uncommon neoplasm of uterus accounting for only 0.2% of all genital tract malignant neoplasms. Outside the uterus, this tumour is extremely rare in the absence of metastasis or extension of a primary uterine neoplasm. Several primary extraterine locations of EES have been reported such as the pelvic cavity, abdominal cavity, ovary, fallo-
pian tube, retroperitoneum and vagina. Extrauterine primary endometrioid stromal sarcomas often arise from endometriosis. Kondi-Pafiti reported on a series of 14 neoplasms arising in endometriosis, with seven cases of ESS among which one case arising in the omentum and another in the pelvis. Endometrial stromal sarcomas (ESS) have been previously divided into low grade and high grade subtypes based on mitotic count by Norris and Taylor in 1966. However, in the WHO classification, the high grade type is replaced by undifferentiated endometrial sarcoma and the distinction between the two entities is based on nuclear pleomorphism and necrosis. Low grade ESS affects younger women with a mean age ranging from 42-58 years, and 10-25% of patients are premenopausal.

On macroscopy, the tumour presents as a solitary well delineated mass. The sectioned surface appears yellow to tan, and the tumour has a softer consistency than a usual leiomyoma. Cystic and myxoid degeneration as well as necrosis and haemorrhage are seen occasionally. In our case, cystic changes were unusually prominent. On microscopy, low grade ESS is usually a dense tumour composed of uniform, oval to spindle-shaped cells resembling the stromal cells of normal proliferative endometrium. Significant atypia and patent pleomorphism are absent. Although most tumours are paucimictotic, mitotic rates of 10 or more per 10 high power fields can be reached. This proliferation of spindle cells is intimately associated with a delicate vasculature of small arterioles that may undergo hyalinization, and thus resemble the spiral arterioles of the late secretory endometrium. Cells with foamy cytoplasm are prominent in some cases. In our case, they were present and intermingled with cholesterol deposits. Sex cord-like structures may also be found. Myxoid and fibrous change may occur focally or diffusely.

ESS with endometrioid glands have been reported in 11 to 40% of published series of ESS. These benign endometrioid glands are focally, sparsely distributed, and their identification depends upon adequate sampling of the tumour. Their presence can be disconcerting if ESS diagnosis is ignored, but of substantial value if the correct diagnosis is suspected. Moreover immunoreactivity for oestrogen and progesterone receptors in ESS is generally seen, with the majority of cases being immunoreactive for both hormone receptors.

The differential diagnosis of a densely cellular spindle cell neoplasm with only minimal cytologic atypia arising in the abdominal cavity of a woman includes all spindle cell neoplasms at this site, but rarely ESS. The absence of endometriosis does not preclude a diagnosis of primary extraterine ESS, as the largest series to date failed to show an association with endometriosis in almost one-half of cases.

This pattern of hyalinization with associated uniform spindle cells is not unique to ESS and can be seen in smooth muscle tumours and in solitary fibrous tumours, but differs from the thicker vessels seen in these two latter entities. The absence of immunoreactivity for CD34 argues strongly against solitary fibrous tumour, while the positive staining for smooth muscle actin and the immunoreactivity for oestrogen and progesterone receptor does not distinguish ESS from a smooth muscle tumour, because both are positive for these antibodies as well as for desmin. CD10 is positive in ESS, while only rare cases of leiomyomas show focal staining. In extra-uterine sites, the other neoplasms included in differential diagnosis are synovial sarcoma, peripheral nerve sheath tumours and gastrointestinal stromal tumour. The immunoreactivity for CD117 and CD34 in gastrointestinal stromal tumour and their absence in ESS are especially useful in this setting. Endometriosis is easily ruled out as it is not consistent with the formation of a distinct large mass. Lastly, metastasis from an ESS of the uterus or a uterine “adenosarcoma” with relatively bland features are to be considered, and the only way to definitely rule out these lesions is to have the entire gynaecologic tract for pathologic review.

The diagnosis of low grade ESS is relevant because this tumour is characterized by indolent growth and late recurrences. The median interval to recurrence is 3-5 years but may exceed 20 years. Pulmonary metastases occur in 10% of stage I tumours. The 5-year survival rate for low grade ESS ranges from 67% to nearly 100% with late metastases and a relatively long-term survival despite tumour dissemination.

In summary, the occurrence of extraterine and extragonadal ESS arising in the abdominal cavity includes a several differential diagnoses. While extremely rare, any spindle cell neoplasm occurring in the abdominal cavity of a female that has bland nuclear features and scant cytoplasm should be considered as a possible ESS, and positive myogenic markers should not exclude its diagnosis. Diffuse strong immunostaining for CD10 confirms the diagnosis of EES in doubtful cases.

A panel of immunohistochemical markers combined with a careful morphologic examination and consideration of ESS in differential diagnosis should allow the pathologist to reach a correct diagnosis in these rare circumstances.


Primary CD30/ALK-1 positive anaplastic large cell lymphoma of the skeletal muscle in a child

M. DRISS, I. ABBES, K. MRAD, S. SASSI, F. OUBICH\*, S. BARSAOUT\*, K.B. ROMDHANE
Department of Pathology, Salah Azaïz Institute, Bab Saadoun, Tunis, Tunisia, Department of Oncology, Children’s Hospital, Bab Saadoun, Tunis, Tunisia

Correspondence
Maha Driss, Department of Pathology, Salah Azaïz Institute, 1006 Bab Saadoun, Tunis, Tunisia - Tel. 0021671577850 - Fax 0021671574725 - E mail: maha.driss@rns.tn

Key words
CD30/ALK+ lymphoma • Anaplastic large cell lymphoma • Soft tissue • Childhood • Non-Hodgkin lymphoma

Summary
Anaplastic large cell lymphoma (ALCL) represents approximately 10 to 30% of all childhood non-Hodgkin lymphomas. It frequently involves both lymph nodes and extranodal sites whereas primary or secondary muscular involvements are quite uncommon. We describe a case of an 8-year-old boy presented with one month progressively swelling right buttock mass without association of lymphadenopathy or skin extension. Biopsy of the lesion showed large anaplastic cells with voluminous and abundant cytoplasm as well as folded nuclei. The tumour cells were positive for CD30, CD3, EMA and ALK-1. Chemotherapy resulted in durable remission status. This case emphasizes the occurrence of anaplastic large cell lymphoma in the soft tissue and the favourable outcome of ALK-positive anaplastic large cell lymphoma.

Introduction
While skeletal muscle can be involved in anaplastic cell lymphoma as a part of disseminated disease, primary anaplastic large cell lymphoma (ALCL) of skeletal muscle is exceedingly rare with only a few cases reported in the English literature\(^1\)\(^2\). We present a case of an 8-year-old boy diagnosed with a CD30/ALK+ ALCL in skeletal muscle, with no other sites of disease; the boy is still disease-free 7 years after completion of chemotherapy. To the best of our knowledge, this is the first paediatric case reported in the literature.

Case report
A previously healthy 8-year-old boy presented in June 1999 with a month-long history of an enlarging painful mass of the right buttock. Initial physical examination was essentially non-informative except for the mass. He had no history of fever, weight loss or other systemic symptoms. Furthermore, neither lymphadenopathies nor skin changes were detected. Laboratory studies showed mild leukocytosis and an elevated erythrocyte sedimentation rate (48 mm). Magnetic resonance imaging study of the pelvis revealed a large soft tissue mass of 8 cm in diameter wrapping the hip, but without extension to the proximal groin. The mass was heterogeneous with low signal intensity on T1-weighted images and high signal intensity on T2-weighted images (Fig. 1). On the basis of these findings, a diagnosis of rhabdomyosarcoma was initially suspected. The patient underwent a tumor biopsy, which revealed anaplastic large cell lymphoma. Further staging studies, which included computed tomography (CT) of the chest and abdomen as well as bone scan and bone marrow aspiration did not reveal evidence of dissemination of disease. In addition to these findings, the patient was diagnosed as having Ki-1 ALCL lymphoma primary in soft tissue. Accordingly, intensive chemotherapy was prescribed as follows: doxorubicin, ifosfamid, cyclophosphamide, adriamycin and intrathecal methotrexate. One month after the completion of the chemotherapy regimen, MRI revealed the complete disappearance of the tumour. The patient has been in complete remission for more than 7 years without further treatment.

Materials and methods
Two small fragments of tissue fixed in 10% buffered formalin were received. The specimen was embedded in...
paraffin, and 4-µm-thick paraffin sections were stained with haematoxylin-eosin. Immunohistochemistry was performed using the following antibodies (Dako; 1/50): cD3, cD20, cD15, cD30, cD45rO, cD68, cD79a, epithelial membrane antigen (EMa), vimentin, desmin, actin, PS100 and aLK-1.

Results

Microscopic Findings
Histological sections revealed striated muscle tissue extensively infiltrated by sheets of large neoplastic cells with irregularly indented embryo-like nuclei, vesicular chromatin, and prominent nucleoli (Fig. 2A). The cytoplasm was abundant amphophilic with distinct cytoplasmic membranes and pale paranuclear eosinophilic regions (Fig. 2B). Mitotic figures were numerous and there were occasional areas with apoptotic features. A variable number of reactive cells such as small lymphocytes, plasma cells and neutrophils were found, including histiocytes, which imparted a “starry-sky” appearance and occasionally showing signs of erythrophagocytosis. One striking finding, however, was the presence of tumor emboli appearing as ill-defined cellular aggregates that showed central loss of cellular cohesion reminiscent of alveolar areas.

Immunohistochemical Findings
All tumour cells were strongly positive for CD30, ALK-1 and vimentin, and were focally positive for EMA, CD3 and CD45RO. Tumor cells were negative for CD20, CD79a, CD68, actin, desmin and S100 protein. CD30, EMA and vimentin staining were localized at the paranuclear regions and the cell membrane. ALK-1 had an obvious cytoplasmic and nuclear pattern of immunoreactivity (Fig. 2C). Histiocytes were positive for CD68, which highlighted imaging of erythrophagocytosis (Fig. 2D).

Discussion
Primary malignant lymphoma of soft tissues is rare, accounting for less than 2% of lymphoma of all sites and is most often of B cell phenotype. It is characterized by the development of a soft tissue mass in a region not
Typically considered rich in lymph nodes and without extension from adjacent skin, lymph node or bone. Furthermore, staging studies must exclude other sites of distant metastases. About 15 to 85% of systemic ALCL contain the translocation (2;5)(p23;q35) which fuses the anaplastic lymphoma kinase (ALK) gene at 2p23 with the nucleophosmin (NPM) gene at 5q35, resulting in the production of a chimeric NPM-ALK protein. The detection of ALK by reverse transcription polymerase chain reaction and by immunohistochemistry has made possible a new classification between ALK positive and ALK negative ALCLs. ALK-positive ALCL occurs earlier in life and accounts for 10 to 30% of all childhood non-Hodgkin lymphoma. It frequently involves both lymph nodes and extranodal sites including the skin, lung, bone, soft tissue and gastrointestinal tract. In contrast to ALK-positive ALCL, ALK-negative ALCL are characterized by a higher age and advanced stage at diagnosis and poor prognosis. It shows similar clinical features to ALK-positive ALCL, but extranodal involvement is less common. Although soft tissue involvement may be seen at diagnosis or relapse in primary systemic ALCL, the sole involvement of skeletal muscle is quite uncommon with only 2 documented cases reported in the international literature. Ishii reported a case of paediatric ALK-positive ALCL in a Japanese girl who presented with an intramuscular tumour on her upper arm. However, further investigation at diagnosis showed an enlarged lymph node. In our case, diagnosis of Ki-1 ALCL was performed by morphological and immunological analysis of the intramuscular tumour. Additionally, we think that the ALCL arising primarily within muscle because the tumour was intimately admixed with muscle fibres. Furthermore, we noted that the tumour was situated far from the proximal groin, and staging studies showed no evidence of disease elsewhere or extension from adjacent skin, lymph node or bone. Immunohistochemically, both cytoplasmatic and nuclear ALK staining strongly suggested that tumour cells contained the 2;5 translocation. Unfortunately, cytogenetic analysis was not possible. The main differential in both clinical and histological diagnosis considered in this case was rhabdomyosarcoma. In fact, the patient’s age as well as the presence of an exclusively intramuscular mass without concomitant lymphadenopathy, associated cutaneous or osseous change was suggestive of rhabdomyosarcoma. Furthermore, the presence of ill-defined cellular aggregates with central loss of cellular cohesion is suggestive of the alveolar area of rhabdomyosarcoma. Such a distinction may be achieved mainly by immunohistochemical stains that include a panel for muscular markers which, as in our case, was non-reactive. Reactive histiocytosis with erythrophagocytosis, as described in our case, is occasionally reported in ALCL and can be incorrectly diagnosed as malignant histiocytosis.

In conclusion, ALCL may be present solely in soft tissue. Despite the overtly anaplastic appearance of this lymphoma, favourable long-term survival is possible, and in our case study the patient is still alive and well 7 years after completion of chemotherapy. The paediatric age and ALK expression may have played an important role in this patient’s excellent post treatment outcome.

References

4. Salamao DR, Nascimento AG, Lloyd RV, Chen MG, Habermann...


Small cell osteosarcoma: a case report

Pathology Department, *Oncology Department, **Radiology Department, ***Orthopedic Department, H. Bourguiba Hospital, Sfax, Tunisia

Correspondence
Dr Rim Kallel, Pathology Department, H. Bourguiba Hospital, 3029 Sfax, Tunisia - Tel. 216 74240341 - Fax 216 74243427 - E-mail: rim.kallel@yahoo.fr

Key words
Small cell osteosarcoma • Round cell tumor • Chemotherapy

Summary

Introduction
Small cell osteosarcoma (SCO) is a rare bone tumour representing 1.3% of all osteosarcomas. This rare variety of osteosarcoma tends to arise in the metaphysis of long bones and may extend secondary to epiphysis. By histopathology, the tumour is composed of small round cells with a variable degree of osteoid production. We report a new case of SCO in the distal femur with epiphyseal involvement. We also present the clinical, radiologic and therapeutic features of SCO with particular emphasis on the pathologic features that allow differentiation of this neoplasm from other small round cell tumours.

Observation
A 14-year-old girl presented with a 6-month history of a painful tumefaction of the left knee. Physical examination revealed a firm mass in the distal left femur with motor deficit; no regional adenopathy was evident. Radiographs of the knee demonstrated an aggressive metaphyseal lytic lesion of the internal femoral condyle with involvement of the epiphysis and cortical destruction and invasion of soft tissues. Histological examination of a biopsy specimen showed sheets of neoplastic small round cells simulating Ewing’s sarcoma. Osteoid was focally present. A diagnosis of SCO was made. The patient received 2 cycles of adjuvant chemotherapy with ifosfamide, adriamycin and cisplatin. MRI showed no change in tumour size. An en bloc, wide-margin resection of the lesion was performed. Histological examination showed a viable tumour with few necrotic foci. The patient received adjuvant chemotherapy with Holoxan and VP16. The clinical response was favourable.

Conclusion
SCO is a rare clinical entity with a high grade of malignancy that must be distinguished from other round cell tumours, particularly Ewing’s sarcoma, in order to optimise treatment protocols.

Introduction
Small cell osteosarcoma (SCO) of the bone is a rare variant of osteosarcoma that poses unique diagnosis and treatment considerations. The tumour arises in the metaphysis of long bone. We report a new case in the distal femur with epiphyseal involvement. We also present the clinical, radiologic and therapeutic features of SCO with particular emphasis on the pathologic features that allow differentiation of this neoplasm from other small round cell tumours.

Case report
A 14-year-old girl presented with a 6-month history of a painful tumefaction of the left knee. Physical examination revealed a firm mass in the distal left femur with motor deficit; no regional adenopathy was evident. Radiographs of the knee demonstrated an aggressive metaphyseal lytic lesion of the internal femoral condyle with involvement of the epiphysis and cortical destruction and a periosteal reaction. Computed tomography (CT) and magnetic resonance imaging (MRI) showed a large tumour in the metaphysis involving the epiphysis of the distal femur measuring 6.5 x 5.5 x 5.5cm with cortical destruction and involvement of soft tissues (Fig. 1). The lesion showed inhomogeneous radiotracer uptake. Skeletal scintigraphy showed strong hyperfixation in the left femoral condyle. Chest radiographs and CT were normal. An open surgical biopsy was collected. Histological examination showed sheets of small neoplastic round cells with scant cytoplasm, round to oval nuclei and a fine chromatin pattern; mitotic figures were abundant; focal areas of tumour osteoid were rarely seen (Figs. 2, 3). By immunohistochemistry, tumour cells were strongly positive for vimentin and neuron specific enolase (NSE), weakly positive for CD99 (cytoplasmic staining) and negative for epithelial membrane antigen (EMA) and leukocyte common antigen (LCA). A diagnosis of SCO was made. The patient received 2 cycles of adjuvant chemotherapy with ifosfamide, adriamycin and cisplatin. MRI showed no change in tumour size.
An en bloc, wide-margin resection of the lesion was performed. The tumour was localized in the metaphysis and extended into the femoral condyle. Histological examination showed a viable tumour with few necrotic foci; surgical margins and the articular cartilage of the epiphysis were uninvolved. The patient received adjuvant chemotherapy with Holoxan and VP16. The clinical response was favourable after a follow-up of 2 years.

**Discussion**

SCO of the bone is rare, accounting for 1.3% of all osteosarcomas. It was first reported by Sim et al. in 1979. It is a tumour of young adulthood, with a reported age range from 19 to 28 years (average, 19 years). There is a slight predilection for females, sex ratio 1.1:4. The anatomic distribution of SCO is similar to that of conventional osteosarcoma: the distal femur is the most common site, followed by the proximal tibia and proximal humerus. The metaphysis of long bones is most commonly affected, followed by a diaphyseal location accounting for 14% of cases of long bone SCO. It was originally suggested that the epiphyseal plate is a barrier to the spread of tumour, as cartilage was believed to contain a substance that inhibits angiogenesis and collagenase formation by tumour tissue. However, some authors have reported invasion of the epiphyseal plate by osteosarcoma. In a study of 11 cases of SCO, Bertoni et al. reported seven cases extending into the epiphysis, and in a study by Norton et al. involvement of the epiphysis was present in 12 of 15 cases (80%). These findings are contrast with the common misconception that the epiphyseal plate is a barrier to tumour spread.
The clinical presentation of SCO at the time of initial diagnosis is similar to that of conventional osteosarcoma. Pain and swelling are common clinical manifestations, often beginning with intermittent pain, swiftly turning into persistent pain. The duration of symptoms before presentation usually ranges from a few weeks to several months.

The radiographic features of SCO are nonspecific, although the tumour shows a predominantly lytic lesion and an aggressive malignant appearance with destruction of the cortex. There is always a lytic component, usually admixed with radiodense areas. The appearance on radiographs of mineralized matrix outside the bone is an indication of osteosarcoma. Reactive bone sclerosis and soft tissue mineralization may also be seen in SCO. MRI is useful for evaluating infiltration of the marrow space, soft tissues and joint spaces, and is also used to assess the extent of transepiphyseal involvement. Because osteosarcomas may present as lytic tumours that may extend into the epiphysis, they should be included in the differential diagnosis of clear cell chondrosarcoma, chondroblastoma, epiphyseal enchondroma and mesenchymal chondrosarcoma.

Histologically, SCO of bone is a tumour constituted predominantly of small cells with variable osteoid production. Specifically, it is composed of round cells or short spindle cell types; the round cells are small, may have a lymphoma-like pattern and have a scanty cytoplasm; nuclei are round to oval and chromatin may be fine to coarse; mitoses range from 3 to 5/high power field. In the short spindle cell type, the tumour cells are mainly composed of short-spindle nuclei containing fine granular chromatin with scanty amounts of cytoplasm. Lace-like osteoid production is always present, but in variable amounts thus requiring an adequate sampling of the tumour. Moreover, reactive periosteal bone formation, fibrin deposits, necrotic debris and stromal collagen may all be misinterpreted as areas of osteoid synthesis by tumour cells. It can be critical for diagnosis if the biopsy material does not clearly include osteoid: this is especially important in SCO, where the small round cells may histologically simulate Ewing’s sarcoma (EWS). EWS occur more frequently, and although reactive new bone may be seen, there is no malignant osteoid. The tumour cells of SCO are more pleomorphic than in EWS and may indeed show spindling of nuclei. A positive PAS staining does not favour a diagnosis of EWS since it is usually positive in SCO as well. By immunohistochemistry, there are no specific reports exploring its use in differential diagnosis between SCO and EWS; CD99, a relatively specific marker for EWS may be positive in some cases of SCO; however, the staining is not membranous, intense and diffuse. In addition, the tumour may be difficult to distinguish from other small round cell malignancies, such as malignant lymphoma and mesenchymal chondrosarcoma. Lymphomas of bone are predominantly composed of larger cells with prominent nucleoli, and it rarely occurs in the first or second decade of life. Furthermore, LCA is useful for differential diagnosis. Primitive neuroectodermal tumour of bone (PNET), which belongs to the Ewing’s family of tumours, can be ruled out as neither bone or cartilage is identified and neuroectodermal markers (neuron specific enolase and Leu-7) are usually positive. Other small cell malignancies can be easily ruled out by immunohistochemical staining (e.g. with keratins in the case of metastatic small cell carcinoma). By cytogenetic analysis, the 11:22 translocation of Ewing’s tumours is not usually seen in this neoplasm, although Noguera has reported this chromosomal translocation in a case of SCO.

Some authors believe that SCO is a definite histological entity. It seems reasonable that tumours, with spindling small cells or when matrix production is definite, should be treated based on a protocol for osteosarcoma. This would include neoadjuvant chemotherapy and surgical removal with a wide margin by amputation or a limb-salvage procedure. If tumour cells are small, round, and uniform and matrix is questionable, it probably is best to treat it as EWS. It has been suggested that SCO may be less sensitive to irradiation than EWS, and that ablative surgery and adjuvant chemotherapy offer the best chance for control of the disease.

The SCO has a poorer prognosis than conventional osteosarcoma (5-year survival rate of 77%) and EWS (5-year survival rate of 50%) and long-term prognosis depends on several factors including size, location, tumour extent, marrow involvement, presence and number of metastases at diagnosis, and response to preoperative chemotherapy.

**Conclusion**

SCO is a distinct variant of osteosarcoma with unique histological features. This tumour must be differentiated from other small cell malignancies because of treatment considerations, particularly patient response to chemotherapy. When dealing with a small cell tumour, it is important that pathologists look carefully for matrix production; in addition, the appearance on radiographs of a mineralized matrix is an indication of osteosarcoma.

**References**


Extraventricular neurocytoma in a child mimicking oligodendroglioma: a diagnostic pitfall

Departments of Pathology and Neurosurgery, La Rabta Hospital, Bab Saadoun, Tunis, Tunisia

Key words
Extraventricular • Neurocytoma • Oligodendroglioma • Central nervous system • Immunohistochemistry

Summary
Extraventricular neurocytomas are rare neuronal tumours that have been included in the 2007 WHO classification as a variant of central neurocytoma. They arise outside the ventricles, usually within the cerebral hemispheres but also in other regions throughout the neuraxis. The morphological overlap of these tumours with oligodendroglioma often poses diagnostic difficulty. Herein, a case of extraventricular neurocytoma in a 4-year-old girl is reported that mimicked histologically oligodendroglioma. The authors describe the clinicopathological features of this rare entity with special emphasis on differential diagnosis.

Introduction
Extraventricular neurocytomas (EVN) are rare neuronal tumours recently included in the 2007 WHO classification as a variant of central neurocytoma. They arise outside the ventricles, usually within the cerebral hemispheres, but also in other regions throughout the neuraxis. When neurocytomas occur in the cerebral hemispheres, where oligodendrogliomas are more common and better recognized, diagnostic confusion often arises due to the striking morphological overlap of both tumours. In this paper, we report a new case of EVN located in the left parieto-temporal lobe of a 4-year-old child that mimicked histologically oligodendroglioma. Our aim was to describe the clinicopathological features of this rare entity with special emphasis on differential diagnosis.

Clinical history
A 4-year-old, previously healthy girl, presented with a one-month history of headache, vomiting and progressive onset of weakness on the right side of her body. On admission, the child was alert and oriented, with normal speech. Neurological examination revealed right hemiparesis with hyperactive reflexes on the right ankle and a negative Babinski response. Pupils were reactive to light and there were no cranial nerve deficits. CT scan showed a hyperdense calcified lesion located in the left parieto-temporal lobe with peri-lesional oedema, significant mass effect on midline structures and central hypodense zones consistent with cystic change (Fig. 1). The lesion did not enhance after contrast medium injection. Subtotal excision of the tumour was achieved. At surgery, the tumour was clearly demarcated from the surrounding brain parenchyma, had a soft consistency and bled moderately. Histological examination of the surgical specimen revealed a proliferation of cells with large, clear cytoplasm and a round or ovoid nucleus embedded in a neuropil-like background (Fig. 2). Some areas showed greater cellularity with limited intervening matrix. Vascularization was rich and there was no evidence of necrosis, haemorrhage, or endothelial proliferation. Mitotic figures were rare. Based on cytologic

Correspondence
Faten Limaem, Department of Pathology, La Rabta Hospital, Bab Saadoun (1007) Tunis, Tunisia - Tel. +216 96 55 20 57 - E-mail: fatenlimaiem@yahoo.fr

Fig. 1. CT scan demonstrating a hyperdense calcified lesion located in the left parieto-temporal lobe with central hypodense zones consistent with cystic change, perilesional oedema and significant mass effect on median structures.
and morphological features, a differential diagnosis including oligodendroglioma and neurocytoma was considered. Immunohistochemical staining for synaptophysin was diffuse and highlighted the neuropil matrix surrounding tumor cells (Fig. 3). Rare tumor cells showed weak reactivity for glial fibrillary acidic protein. Labeling with Ki-67 (Mib-1) yielded a tumor cell proliferation index as high as 1%. Given these morphological and immunophenotypic features, a diagnosis of extraventricular neurocytoma was rendered. No adjuvant radiation therapy was administered post-operatively. At the last routine follow-up visit (1 year post-operatively), the patient complained of recurrent headaches. CT scan demonstrated tumor recurrence and the patient underwent further tumor resection. Histological examination of the surgical specimen showed no features suggesting that the tumor had undergone progression or contained additional elements. At present, the child is still on follow-up and has shown no evidence of recurrence.

**Discussion**

Neurocytomas are rare tumors of the central nervous system constituting approximately 0.25-0.5% of all intracranial tumors. The definition of “central neurocytoma” is restricted to neoplasms located within the ventricles, whereas the term “extraventricular neurocytoma” is used for neurocytomas arising at other sites such as the cerebral hemispheres, thalamus, amygdala, pineal gland, retina, skull base, cerebellum, pons, spinal cord and cauda equina. To our knowledge, about 60 cases of EVN have been reported in the literature to date, with the frontal and parietal lobes being the most commonly affected sites. Neurocytomas, either at ventricular or extraventricular locations, commonly affect young adults with no gender predilection. Our patient was a 4-year-old female child. Patients with EVNs usually present with seizure or hemiparesis. In the presented case, the chief complaints were right hemiparesis, headache and vomiting. On CT scan, cerebral neurocytomas have been described as hypodense or isodense relative to grey matter, and as for intraventricular neurocytomas, coarse or punctate calcification is common. In the present case, CT scan showed a spontaneously hyperdense, calcified lesion located in the left parieto-temporal lobe with hypodense zones consistent with cystic change. The radiological finding is somewhat different from those described to date. On MR images, cerebral neurocytomas appear hypointense on T1-weighted sequences and hyperintense on T2-weighted studies. Contrast enhancement is mild and occasionally rim-like. Extraventricular neurocytomas often pose a diagnostic challenge because of overlap of clinical, radiologic and histological features with those of other primary brain tumors, particularly oligodendroglioma. Although pediatric oligodendrogliomas are rare, they must be differentiated from neurocytic tumours. One critical distinction between oligodendroglioma and EVN lies in their respective interactions with adjacent brain. Whereas oligodendroglioma is diffusely infiltrative, EVN appear rather circumscribed. Extraventricular neurocytomas often pose a diagnostic challenge because of overlap of clinical, radiologic and histological features with those of other primary brain tumors, particularly oligodendroglioma. Although pediatric oligodendrogliomas are rare, they must be differentiated from neurocytic tumours. One critical distinction between oligodendroglioma and EVN lies in their respective interactions with adjacent brain. Whereas oligodendroglioma is diffusely infiltrative, EVN appear rather circumscribed. Histologically, EVNs demonstrate pushing borders with respect to surrounding brain. While the presence of infiltrated CNS axons can be demonstrated in oligodendroglioma by neurofilament immunohistochemistry, this is less evident in EVN. The aforementioned properties are particularly significant because the cytological features of oligodendroglioma and neurocytoma overlap and cannot always be relied upon for distinction. Both EVN and oligodendrogliomas predominantly contain a clear cell population of well-differentiated cells with round, regular nuclei. Neither contains the course fibrillarity of astrocytic differentiation, but EVN often show a delicate neuropil background. Ganglionic differentiation, if present, strongly favors EVN. EVNs are strongly positive for neuronal markers, such as synaptophysin, and show only focal staining for glial...
fibrillary acid protein (GFAP). Neuronal differentiation is necessary, but not sufficient for the diagnosis of EVN, since oligodendrogliomas, surprisingly, can also show some neuronal differentiation by immunohistochemistry and ultrastructural studies. The morphological overlap between EVN and oligodendroglioma, the lack of specific oligodendrogial markers, and the co-expression of neuronal markers in EVN and oligodendroglioma have all provided evidence that oligodendrogial and neurocytic lesions may form a biological and diagnostic spectrum. The ends of the spectrum are anchored by classical oligodendroglioma and EVN, but rare tumours with intermediate or biphasic differentiation would be expected. Recent investigations have attempted to provide genetic markers that can distinguish classic EVN from oligodendroglioma. An obvious place is the status of chromosome 1p and 19q, since their combined loss is frequent in a large subset of oligodendrogliomas and has been shown to be prognostically significant. A recent fluorescence in situ hybridization (FISH) analysis of oligodendroglioma and its mimic found that 70% of oligodendrogliomas showed losses of 1p and 19q. Unexpectedly, infrequent losses of 1p and 19q (16%) were also seen in EVN, further clouding the distinction between EVN and oligodendroglioma. On the other hand, Kreiger et al reported that paediatric oligodendrogliomas rarely demonstrate losses of 1p and 19q, suggesting that they have a distinct molecular pathogenesis. These findings argue that 1p and 19q loss cannot be used as a molecular diagnostic marker for oligodendrogliomas in children, and raise the challenge of identifying other genetic alterations that might characterize these tumours. Like central neurocytoma, most EVNs do not recur, especially after complete resection. Subtotal resection, high proliferation rates, atypical histological features and older patient age appear to be associated with an increased likelihood of recurrence. Our patient developed tumour recurrence due to subtotal excision of the primary tumour.

References