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"The existence of lymphatic capillaries influences lymphocyte recirculation and immune cell movement through the interstitium of connective tissue in peripheral organs" (e.g., intestine, skin, kidney and others) (see page 762)

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The Israel Medical Association Journal (IMAJ) is the successor of *the Israel Journal of Medical Sciences* that was initiated in 1965 by Prof. Moshe Prywes. During the 32 years of publication. Prof. Prywes served as its editor-in-chief.

A Woman and Her Son in a Ma'abara
Leo Roth
Oil on canvas, 64 x 54 cm
Courtesy of Tiroche Art Gallery

Leo Roth was born in 1914 in the Jewish village of Tismanitza in Galicia. The family moved to Germany and in 1930 he began his studies at the School of Art in Hamborn under the direction of the artist Josef Doppelfeld. In 1933 Roth left Germany for Palestine, settling in Tel Aviv. After visiting relatives on Kibbutz Kinneret he decided to join Kibbutz Afikim in the Jordan Valley. In his early years he worked as a shepherd and later was put in charge of decoration of the kibbutz dining room and of scenery for kibbutz productions. He continued painting. After studying fresco and mural painting at the Ecole des Beaux Arts, Paris, he was appointed director of the Art Academy established by the Kibbutz movement. His works appeared in numerous solo and group exhibitions in Israel and abroad. Leo Roth died in 2002.

In the artist's words: "A creative artist is endowed with memories and experiences stemming from his forefathers and generations past. This heritage obliges him to be their voice. Yet his creation and whatever he does is guided by superior forces beyond his control. All he is able to do, then – his duty throughout his life and work – is to learn to improve his craft and performance."

The picture shown here reflects a period in the history of modern Israel. *Ma'abarot* (plural of *ma'abara*) were refugee absorption camps in the 1950s intended to provide accommodation for the large influx of Jewish refugees and immigrants arriving to the newly independent State of Israel, replacing the less habitable "immigrant camps" or tent cities. The new arrivals were mainly from the Middle East and North Africa, but there were also Holocaust survivors from Europe. The conditions in the Ma'abarot were very harsh, with many people sharing sanitation facilities. Over time, the Ma'abarot metamorphosed into Israeli towns or were absorbed as neighborhoods of the towns they were attached to, and residents were provided with permanent housing.

Physics, Biology and the Origin of Life: The Physicians' View

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ABSTRACT: Physicians have a great interest in discussions of life and its origin, including life's persistence through successive cycles of self-replication under extreme climatic and man-made trials and tribulations. We review here the fundamental processes that, contrary to human intuition, life may be seen heuristically as an ab initio, fundamental process at the interface between the complementary forces of gravitation and quantum mechanics. Analogies can predict applications of quantum mechanics to human physiology in addition to that already being applied, in particular to aspects of brain activity and pathology. This potential will also extend eventually to, for example, autoimmunity, genetic selection and aging. We present these thoughts in perspective against a background of changes in some physical fundamentals of science, from the earlier times of the natural philosophers of medicine to the technological medical gurus of today. Despite the enormous advances in medical science, including integration of technological changes that have led to the newer clinical applications of magnetic resonance imaging and PET scans and of computerized drug design, there is an intellectual vacuum as to how the physics of matter became translated to the biology of life. The essence and future of medicine continue to lie in cautious, systematic and ethically bound practice and scientific research based on fundamental physical laws accepted as true until proven false.

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There are few of us, especially practitioners of medicine, who after an encounter with birth or death do not come away, no matter how tired, with wonderment at the origin and significance of these two events. With age, we become more involved in philosophical issues of death and in the broader issue of whether we are alone in our galaxy. Medieval doctors were often philosophers in the wider sense of their own time/environment and were very much concerned with the inseparable issue of 'what' is life and 'how' it relates to inanimate matter. Physicians of those eras were more concerned about religion and the role of man and God than about physical science. One wonders what they would think today, with physical science dominated and puzzled by the apparent incompatibility between two fundamental concepts: general relativity (gravitational) theory that views matter-energy as an expression of a time-space continuum and quantum theory that considers matter-energy in terms of discrete quantities (quanta) and waves and their counterintuitively weird behavior. The long-held desire to unify these two fundamental laws of physics in a theory of everything remains a mirage [1].

However, we cannot escape the physical nature of life and the direct relationship of genetics, the brain and the life sciences in general, with the dualities of mass/energy and gravitational continuum/quantum discretion, thus not only with chemistry but also with the fundamental physics of the universe. Indeed, in 1946 it was proposed that the transfer of genetic information in discrete bits evidences a quantum mechanical world [2]. Just as physicians of today quickly took to the principles behind MRI (magnetic resonance imaging) and PET (positron emission tomography) imaging, they will not be troubled by the finding that a single photon of radiation passes at one and the same time through each of two slits in an opaque barrier placed transverse to its trajectory, and that the waves associated with that photon after it has passed through the slits interfere with each other, as seen on a suitable screen. Yet, the photon itself is detected only as a single spot on the screen. Such counterintuitive principles are not black magic, nor are the pairs of directly unobservable, 'virtual' subatomic particles that are thought to come into existence in a vacuum, but may then immediately annihilate each other. As principles of modern physics are absorbed increasingly into such fields as clinical imaging, neurophysiology, behavioral psychology, protein crystallography and drug design, they also enlighten the ever-present wonder on the nature of life.

WHAT IS LIFE?

There is no shortage of definitions of life [3]. Often, scientists quote the terse definition adopted by NASA in 1994: a self-

contained chemical system capable of undergoing Darwinian evolution. A compendium of current thought could be that an entity is 'live': a) if it replicates through successive generations of physical embodiment energized by thermodynamically favorable metabolic processes, b) if the store of information on which the replication and processes depend is inherent in and propagated together with the embodiment, and c) if random changes in the store of information are manifested in the embodiment and increase its chance of surviving a changing geophysical environment and challenges from other self-replicating internal or external entities.

Life may be discussed heuristically as a fundamental process in the universe at the interface between the complementary forces of gravitation and quantum mechanics

Overall, life as so defined is most favored selectively when the rate of random change in a replicating information store is most suited to the rate of change in the different challenges faced. A too rapid change in challenges, e.g., a new virus spread by air travel, may catastrophically overwhelm adaptation of immunity. At the same time, although a relatively stable information store and its physical embodiment may best survive minor challenges, a rapidly randomizing store may best survive extreme change, e.g., that caused by wind-spread irradiated dust from nuclear explosions. What universal fundamentals stand behind life and its survival?

PHYSICS AND THE PHYSICIAN

TOWARDS MODERN PHYSICS

There are multiple advances in physics that have had unique medical applications; some of these are highlighted in Table 1. For example, in the late 17th century, Newton propounded laws universally relevant to the motions of matter such as the planets and apples, established the spectral nature of light, considered it to be corpuscular without explanation, and built the first reflecting telescope, while believing matter to be transmutable by alchemy. In 1827, Young and Fresnel demonstrated that light had a wave-like quality but could not explain it. In 1856, following the remarkable experimentation of the barely educated Faraday, Maxwell confirmed mathematically that electricity and magnetism are related phenomena, together constituting electromagnetic waves radiating in all directions at a constant speed, e.g., visual light [4-7]. Then in 1895, Roentgen discovered that non-visible radiation could reveal internal details of living bodies. These findings were the beginnings of the revolutions that led to the marriage of science, technology and medicine.

BIRTH OF QUANTUM PHYSICS

In 1900, Planck described laboratory findings in which the rise or fall in the temperature of a heated chemical element was continuous, whereas the frequency of the wavelengths of light emitted from it changed discontinuously by integral multiples of a mathematical constant. However, he regarded "his constant" only as a convenience, not the signature of a fundamental physical law that assigns a particulate property to light. In 1905, the statistical treatment by Einstein of Brownian motion (the quivering of very minute objects such as pollen in a liquid medium) suggested that the pollen was moving continually under the impact of unseen particles, i.e., matter too could be considered in terms of discrete subdivisions [8]. Experimentation soon confirmed that such motion demonstrated the existence of atoms and molecules:

Table 1. Physics and medicine

Year(s)	Scientist	Contribution	Utility
1672	Newton	The spectral nature of light	Newton's rings in crystalline lenses
1827	Young	The wave quality of light	Optical aberration
1895	Roentgen	Non-visible radiation revealing internal detail of some living bodies	Radiographic evaluation of pathologies
1905	Einstein	Electromagnetic radiation occurs in packets (photons), i.e., is particulate as well as wavelike explaining Planck's 'quantum' constant and the photoelectric effect	Clinical imaging, irradiation in photo-dynamic therapy and for neonatal hyperbilirubinemia, laser surgery, bedside electronic instrumentation
1909	Rutherford	Discovery of the atomic nucleus following on from earlier work on atomic decay and its rate	Nuclear medicine
1925-1927	Heisenberg	The more precisely one of la pair of related variables is measured (e.g., position and momentum of an electron in an atom), the more indeterminate is parallel measurement of the other (Uncertainty Principle); a related postulation that during measurement of variables of motion, the result only exists at the moment of observation and depends on randomly fluctuating probabilities	Quantum mechanical considerations in study of brainfunction, consciousness, cognition and behavior and related pathologies and therapies
1926	Schrödinger	Specific atomic qualities combined in an equation expressing the waves of electrons orbiting the atomic nucleus as a smeared out, continuous 3D 'cloud'	Basic tool in study of configuration, bonding and reactions in molecular biochemistry
1928	Dirac	Electrons can carry negative or positive charges, implying existence of a 'positive' ('anti-') electron (observed in 1932 and named 'positron), thus initiating the concept of 'anti-matter' and anticipating that collision of a positron with an electron (both have the same mass) annihilates both, producing gamma photons	Positron emission tomography (PET) employs injected radionuclide that emits positrons that, on annihilation with electrons, produce gamma photons for 3D internal imaging

decisiveness after a millennia-long argument about matter's divisibility [6].

Despite the enormous leap in physics due to the earlier elucidation of basic physical laws of light, the science of physics was stood on its ears in 1905 by two novel concepts. In the first, Einstein demonstrated that energy and mass interchange in a fixed ratio dictated by the constant speed of electromagnetic radiation in a vacuum. In the second, Einstein postulated that electromagnetic radiation is particulate and occurs in "packets" or quanta, later termed photons [4-8]. This explained the previously mysterious experimental finding that emission of electrons from a light-irradiated material, e.g., a metal plate (the photoelectric effect), is related to the frequency of the irradiation, i.e., its energy, and not to its intensity. This divisibility of energy into photons, now regarded as particulate as well as wave-like, underlies all clinical imaging. The quantum revolution was then disturbed by another postulation by Einstein: the general theory of relativity (in 1916), reflecting gravity as a geometric property of unified space time, curvature of which is directly related to universal mass and radiation [4-7,9]. The theory has not been refuted to date by experimental and other phenomena, including the expanding scale of modern cosmology; neither has it been reconciled with the quantum theory, which has continued to develop and receive experimental confirmation.

Enormous advances in clinical medical science, including integration of modern technology, have depended on increased understanding of fundamental processes of physics and their translation to biology

THE NEW (ORTHODOX) QUANTUM PHYSICS

Following Rutherford's discovery in 1909 that atoms have a nucleus, in 1913 Bohr explained the separation between observed spectroscopic peaks by suggesting that the electrons in an atom are restricted to closed orbits around its nucleus and that movement of electrons between orbits takes place as discrete, instant 'jumps' from one orbit to another. These jumps require a gain in energy of the electron (by photon absorption), or loss (photon emission), depending respectively on whether the jump is to a higher or lower orbit. In 1924, de Broglie demonstrated mathematically that not only the electron but all subatomic particles have wave as well as particle characteristics and that the length of the closed standing electron wave around a nucleus is an integer multiple of its wavelength. It was soon recognized by Heisenberg that matrices of measurements of variables, such as position, momentum and energy of an electron and of all other subatomic particles, enable a quantitative analysis of their motion and the more precisely one of a related pair of such variables is measured (e.g., the position and energy of an electron in an atom), the less precise is measurement at the same time of the other. Moreover, the difference in precision is always a certain minimum [4-7]. Such limitation on experimental determination prevents knowledge of the future of a particle, an unpredictability that has acquired the label, Uncertainty Principle (not a law).

In a different approach, Schrödinger in 1926 combined specific atomic characteristics in an equation that represented the waves of electrons orbiting the atomic nucleus as a continuous 3D "cloud." This approach, more intuitive than matrix maths, quickly enabled a deeper understanding of chemical bonding in terms of the overlap of electron clouds. However, discontinuity was soon emphasized again when Born adapted the wave equation to statistical analysis of the distribution of the probabilities of finding an electron. Then, two more novel far-reaching quantum mechanical propositions added support for quantum unpredictability: during measurement of the variables of motion, the observer influences the result, and the result only exists at the moment of observation [4-7]. Thus, prediction of future material events depends on randomly fluctuating probabilities and potentialities: a motivation for mystical incursion into scientific debate, biology and thereby medicine [10-13]. Indeed, despite eventual basing of quantum mechanics on a rigorous mathematical foundation [14], some now orthodox quantum concepts would have long ago lost support if they had not accurately substantiated otherwise unexplained atomic and sub-atomic experimental data. Consequently, most though not all physicists have continued to deny causality, one of the most obvious of human intuitions.

Nevertheless, supporters of the older, 'classical' quantum theory continued to claim that denial of the objectivity of natural physical properties is a result of the incompleteness of quantum theory as a whole [8,15]. Moreover, although matrix mechanics stipulate that at the moment an atomic electron is precisely located (observed) its wave quality and probability distribution are momentarily abolished ('collapse'), data on atomic electrons were found by Dirac to be equivalent whether calculated by wave equation or matrix math [4-7]. However, although the meaningfulness of the 3D wave nature of a particle and its associated wave function have not been abolished by quantum 'uncertainty' and observational subjectivity, phenomena even stranger than momentary disappearance of wave quality haunt human intuition.

QUANTUM WEIRDNESS

Consequences of quantum mechanics are increasingly being considered on the cosmological scale [1,16]. For example, if pairs of quantum particles mentioned above as appearing in vacuo are not annihilated, they can be 'entangled'. Though according to the Pauli exclusion principle that paired sub-atomic particles cannot be in the same quantum state, under quantum theory they can have both their states in the same event superposed on both of them [8]. Consequently, later measurements of properties of one of the particle pair will be instantly affected by a

characteristic of its 'entangled' twin, even if it is many millions of miles away. This troubles human intuition, and its instant nature controversially seems to contradict relativity theory that nothing, mass or information, can travel faster than light.

QUANTUM AND LIFE

Do 'weird' aspects of quantum mechanics, including energy tunnelling, electron spin flip, particle superposition and entanglement and virtual particles, participate in life? Though some such phenomena are already realized in electronic and clinical technology, thoughts such as "...biogenesis as a phase transition analogous to bubble nucleation in quantum field theory," and "... the nucleated lower-energy state as a community of interacting replicators occupying a mesoscopic region of a condensed matter system" [17] may be of little interest here. However, with the origin of life in mind, a view that life is "... such an exceptional state of matter that its formation from an arbitrary initial state would be extremely improbable if quantum mechanics did not drastically speed up the route from matter to life through the parallel processing allowed by quantum superposition...," does at least suggest a solution to how life could develop within the currently accepted time frame of the universe [17]. This is controversial. Indeed, it may be wondered how superposition of sub-atomic states can distinguish and select a 'living' from among an infinity of 'non-living' states – a teleological nightmare.

The essence and future of medicine depends on a systematic and ethically bound practice of scientific research based on fundamental physical laws

There are those who believe that the enigmas of quantum mechanics may account for the differences between live and inanimate matter [18-20] and may have enabled life to emerge directly from the atomic and molecular world as a self-replicating information storage and processing system [17]. Yet, even if living matter does process information quantum mechanically at the biomolecular or cellular/neuronal levels [21,22], an overall view of biology as interaction between information processors [17] seems to regard life itself as 'information'. Though 'information' is fashionable, alone it is as accessible as a list in a locked drawer and as useful as a book read in the dark. The significance of information is in its application. Without physical embodiment, metabolic machinery, and self-initiation, i.e., without an independent dynamic content, information seems as 'alive' as a thinker who can no longer think. As does "intelligent design," information as 'life' begs a question: can current physics lead to understanding of what constitutes life, in the same way that a description of sub-atomic spectroscopic lines can be related to a fundamental law of physics? Many will continue to prefer that non-mystical approach to biological science.

QUANTUM BIOLOGY

Modern quantum physics has found a still relatively limited role in biology. More commonly this is in the study of protein-associated light gathering molecules (e.g., in photosynthesis and vision) and of excited electronic states (e.g., in energy transfer and proton chemistry). Here, of particular interest is a quantum mechanical approach to a relatively simple chemical structure, the peptide bond [23]. However, quantum application to complexity of intertwined nucleic acid chains and of genes is still in its infancy as it regards the interplay between genes and soma and the awesome, endless complexities of immunity. On the other hand, modern physics has notably been called on for interpretations of the human brain.

THE QUANTUM AND NERVE SIGNALLING

A relatively cautious view is that understanding of brain activity needs knowledge of currently unknown physical laws [24] concerning definable physical issues. Thus, one model posits closely spaced, quantum mechanical molecular condensations in the microtubule structures well known to extend in and along neurons [25]. The condensations are regarded as stable for a microperiod long enough for physiological information transfer over distance to synapses crossed by quantum tunnelling, thus influencing brain activity regionally or as a whole [26]. The stability is proposed to be protected from randomizing, room-level thermal influences by the aqueous environment surrounding the microtubule framework [27]. Though controversial [28], the model exhibits how a non-mystical quantum approach to nervous activity is possible, as it is also to wider questions on life and its origin.

THE ORIGIN OF LIFE

A common view is that life 'emerges' rather like a rabbit from a hat, on earth, in space, or both. 'Emergence' pedestals life as a secondary, not fundamental property of the universe. Thus, earliest thinking was on observed emergence from pupae, cast skins and the womb and, prior to the microscope and Pasteur, from spontaneous generation: origins no more likely than the emergence of Venus from the sea. Whether 'emergence' of life is humbling, 'ascent' through natural selection (Darwin referred to descent), modern physics suggests a less emotive, more fundamental status for life. Here, life is conceived as an ab initio, universal manifestation of quantum/gravitational influence predating earth's formation. Yet neither gravity post-Einstein nor quantum wave/particle probabilities and subjective observation have clarified how and where life as we know it originated, whether only on earth, elsewhere or both.

LIFE'S LOCATION

Since spontaneous generation was disproved, it has been asked: which came first, chicken or egg? How could information storage and application, for systematic biological replication

enabling earthbound adaptation to environmental change, precede the machinery of cell metabolism? Many theories have required the presence of one or more of an earthly chemical 'soup', cold ocean depths, hot volcanic vents [3], or mineral templates [23,29-31]. Moreover, under conditions assumed to have been present on primeval earth, experimental synthesis and spontaneous self-assembly from substrates have produced many of the essential amino acids and their polymers, nucleobases (e.g., adenine and thymine), nucleotides and other organics [32-36]. However, the origin of the remarkable left-handed chiral dominance of amino acids remains unsolved despite numerous theories, including recent ones [30,37].

Irrespective of diluteness of the baryonic matter, a thermodynamic equilibrium unfavorable to life's order and uncertainties mentioned above, overall the likelihood is that life did not start on earth but developed with the universe, prior to earth's existence. This would allow immensely more time for life's development than the four-plus billion years of earth's existence, and the possibility of initiation may have increased due to an earlier Hubble universe with a greater density. A smaller universe may also have eased transfer from the earliest locations of life to galaxies across the universe. A further argument for life's spatial origin unrelated to time is that despite cosmological uniformity observed on the large scale, the apparent overall asymmetry of the universe may be the source of ubiquitous biological homochirality [38].

More general is a theory proposing how in a non-randomly evolving universe, spontaneous self-organization can develop from a random starting point into the origin of life [39]. Teleological non-random randomness? If indeed the origin of life is entailed with development of the universe itself, randomness of starting points seems amorphous in contrast to specific events 'non-random' per se and to causally clear outcomes of universal materiality and ambient conditions. Similarly preferable to statistically improbable happenings, will be natural, observable outcomes of interplay between gravitational and quantum mechanics expressed at more than one time or location in the universe. Such could be, for example, a primordial ribosome developing well before earth's formation.

SEARCH FOR LIFE IN SPACE

There are more than a trillion galaxies, each with a trillion stars of which approximately 10% are thought to have planets. To estimate the likelihood of life on a particular planet, the so-called Drake Equation [40] calculates the relationships between stars, planets, time and intelligence. Notwithstanding a number of negative factors, there is a high likelihood of life in each galaxy. The Milky Way disk galaxy, ours, is approximately 100,000 light years in diameter and 1000 light years thick. From assumptions about existing civilizations, it was estimated that in 30–40 million years our galaxy could be populated. However, as the Drake Equation sums multiplication between many approximations, it does not reliably support or deny extraterritorial alien life.

Given the age of the Milky Way, it seems that aliens should have reached earth long ago. Where are they? Sadly, some authors have proposed that a civilization with nuclear energy is invariably doomed. However, whether conjectures about the origin of life, its location and future are nurtured by 'weird' science or mysticism, natural philosophers of medical science of our times will continue to take a special interest in life's origin, while ensuring that the essence and future of medicine continues to lie in cautious, systematic and ethically bound practice and scientific research based on fundamental physical laws accepted as true till proved false.

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Capsule

A human memory T cell subset with stem cell-like properties

Immunological memory is thought to depend on a stem cell-like, self-renewing population of lymphocytes capable of differentiating into effector cells in response to antigen re-exposure. Gattinoni et al. describe a long-lived human memory T cell population that has an enhanced capacity for self-renewal and a multipotent ability to derive central memory, effector memory and effector T cells. These cells, specific to multiple viral and self-tumor antigens, were found within a CD45RO–, CCR7+, CD45RA+, CD62L+, CD27+, CD28+ and IL-7Rα+ T cell compartment characteristic of naive T cells. However, they expressed large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and showed numerous functional attributes distinctive of memory cells. Compared with known memory populations, these lymphocytes had increased proliferative capacity and more efficiently reconstituted immunodeficient hosts, and they mediated superior antitumor responses in a humanized mouse model. The identification of a human stem cell-like memory T cell population is of direct relevance to the design of vaccines and T cell therapies.

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Eitan Israeli

Capsule

Tumor suppressor BRCA1 epigenetically controls oncogenic microRNA-155

BRCA1, a well-known tumor suppressor with multiple interacting partners, is predicted to have diverse biological functions. However, so far its only well-established role is in the repair of damaged DNA and cell cycle regulation. In this regard, the etiopathological study of low-penetrant variants of BRCA1 provides an opportunity to uncover its other physiologically important functions. Using this rationale, Chang et al. studied the R1699Q variant of BRCA1, a potentially moderate-risk variant, and found that it does not impair DNA damage repair but abrogates the repression of microRNA-155 (miR-155), a bona fide oncomir. Mechanistically, we found that BRCA1 epigenetically represses miR-155 expression via its association with HDAC2, which deacetylates histones H2A and H3 on the miR-155 promoter. We show that overexpression of miR-155 accelerates but the knockdown of miR-155 attenuates the growth of tumor cell lines in vivo. Our findings demonstrate a new mode of tumor suppression by BRCA1 and suggest that miR-155 is a potential therapeutic target for BRCA1-deficient tumors.

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Eitan Israeli

Fetal Alcohol Spectrum Disorder in Israel: Increased Prevalence in an At-Risk Population

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ABSTRACT: **Background:** Maternal exposure to alcohol during pregnancy can lead to a wide range of clinical manifestations in their offspring, termed fetal alcohol spectrum disorder (FASD). In Israel, relatively few cases of FASD have been diagnosed and the prevalence has not been systematically evaluated.

Objectives: To determine the number of children with FASD or at risk for FASD in a select population of high risk patients seen at a clinic evaluating foster and adopted children.

Methods: Israeli children under 2 years old who were candidates for domestic adoption or in foster care were prospectively evaluated for clinical manifestations of FASD, and information was obtained regarding parental use of alcohol or other illicit drugs.

Results: Of the 100 patients prospectively evaluated, 8 had mothers with a known history of alcohol consumption during pregnancy. Two of the children had fetal alcohol syndrome (FAS) without known maternal exposure to alcohol and two had partial FAS. Eleven other children were at risk for development of one of the diagnostic categories of FASD.

Conclusions: In a population of pre-adoption and foster children, 15% either had manifestations of FASD or were at risk for developing FASD. Although this is a select high risk population, the data from this study strongly suggest a greater prevalence of FASD than previously assumed. Under-diagnosis of FASD is detrimental to affected children who could benefit from interventions designed to meet the needs of FASD victims.

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KEY WORDS: adoption, alcohol, birth defects, fetal alcohol syndrome (FAS), fetal alcohol spectrum disorder (FASD)

Fetal alcohol spectrum disorder is the leading cause of acquired developmental delay in the world [1]. FASD is caused by ethanol consumption throughout pregnancy, especially during the first trimester. Since ethanol is a teratogen, fetal cellular exposure to this toxin causes birth defects associated with dysmorphic features (e.g., smooth philtrum, thin upper lip, small palpebral fissures, upturned nose, microcephaly), other birth anomalies, neurodevelopmental abnormalities, and neuroanatomic defects [1,2]. The effects of in utero exposure of the fetus to alcohol are long term and are associated with developmental disabilities, cognitive impairments, psychiatric disorders, and social maladjustment [2,3]. The diagnosis of FASD is important for the patient and family since early intervention, appropriate medical follow-up, and social support are crucial for improving outcome and quality of life of both the patients and their families [4].

FASD is highly prevalent in many countries for which statistics are available. It is estimated that the prevalence of FASD among younger schoolchildren may be as high as 2%–5% in the United States and some West European countries [5]. In an Italian study the rate of FASD reached 4.1% in children attending 25 primary schools in the Lazio region [6]. Based on epidemiologic studies, risk factors for having a child with FASD have been identified; these include mothers in their teenage years, single mothers, previous children with FASD, and adopted children from Eastern Europe [7].

The number of children with the diagnosis of FASD in Israel is extremely low and there are no registries or databases in Israel listing children suspected of having FASD. Senecky et al. [8] reported that during a 10 year period (1998–2007), only six patients with a diagnosis of FASD were listed in two of the four health insurance funds in Israel. This may reflect a true low incidence, a lack of awareness among health professionals regarding the diagnostic features of FASD, the absence of systematic data collection in Israel related to FASD, confusion regarding the updated diagnostic criteria of FASD as related to classical fetal alcohol syndrome, or unawareness of the importance of listing FASD as a diagnostic entity [8]. With regard to the possibility of FASD being rare in Israel, this would be quite unlikely given the increase in alcohol consumption in the country [9], a lifestyle among a large segment of the population that is similar to western countries where the prevalence of FASD is high [10,11], and lack of awareness on the part of expectant mothers and adolescent girls regarding the minimal amounts of alcohol that are considered toxic [12,13].

We investigated a group of children at high risk for FASD. The Medical Unit for Adoption and Foster Care at Hadassah

***Dr. Tenenbaum and Dr. Wexler contributed equally to the study**

FASD = fetal alcohol spectrum disorder

Medical Center evaluates Israeli infants and children referred by the Child Services Unit of the Ministry of Welfare and Social Services who are in foster care, are residing in institutions, are candidates for adoption or were recently adopted. We evaluated this group to determine the level of fetal exposure to alcohol and the frequency of children having clinical manifestations consistent with FASD.

PATIENTS AND METHODS

The first 100 children under the age of 2 years who were referred to the medical adoption unit of Hadassah Mount Scopus for comprehensive medical and developmental assessment were prospectively evaluated regarding epidemiological and clinical manifestations of FASD. Information collected during the evaluation was recorded in the patient's medical record and subsequently collated. The study was approved by the Hadassah University Medical Center Institutional Review Board.

All children were examined by a physician, a psychologist and a nurse. The infants' medical and developmental condition and their anthropometric measurements were recorded. The medical history of the child and the biological parents was obtained. However, for many of the patients, complete information was not available either because the child had been abandoned or the parent(s) were not forthcoming with the information. As part of the evaluation, children underwent testing for human immunodeficiency virus, hepatitis, syphilis and, when indicated, other congenital infections. For children with microcephaly or cranial malformations, we recommend a neurologic evaluation and a cranial ultrasound. In nearly all cases follow-up in a child developmental center is recommended.

The diagnostic criteria for FASD and clinical manifestations associated with FASD were based on the U.S. Institute of Medicine's diagnostic classification with modifications based on a Canadian Task Force [14,15]. In brief, the diagnostic categories included:

- Fetal alcohol syndrome with known maternal alcohol exposure and with the child displaying typical dysmorphology associated with FAS (i.e., flat upper lip, flattened philtrum, midline facial hypoplasia), evidence of growth retardation, and central nervous system neurodevelopmental or neuroanatomic abnormalities
- FAS without confirmed maternal alcohol consumption with all the criteria mentioned above
- Partial FAS with confirmed maternal alcohol consumption
- Alcohol-related birth defects
- Alcohol-related neurodevelopmental disorders showing evidence of both neurodevelopmental and social/behavioral abnormalities.

FAS = fetal alcohol syndrome

RESULTS

Demographic data for children and patients are shown in Table 1. For 8% of the mothers there was a known history of alcohol consumption. Most likely this is an underestimation since information regarding alcohol exposure during pregnancy was not available for many of the mothers. This is even more true for the fathers as their identity was often unknown. As seen in Table 1, many mothers are involved in high risk behaviors such as drug use and promiscuity (e.g., prostitution, multiple partners). The median age for the children in our study population was 4.5 months and the mean age 5.9 ± 5.2 months at the initial visit. Almost all the children were evaluated prior to their first birthday.

The clinical characteristics of the children are listed in Table 2. Of the children with documented exposure to alco-

Table 1. Demographic characteristics of patients and biological parents

Patient's age (mos)	
Mean (SD)	5.9 ± 5.2
Median	4.5
Range	.5–24
Gender	
Males	42
Females	58
Religion of parents	
Jewish	59
Moslem	16
Christian	9
Mixed	13
Unknown	3
Maternal history	
Alcohol use	5
Drug use	14
Both	3
Promiscuity	3
Paternal history	
Alcohol use	0
Drug use	6
Both	2

Table 2. Clinical characteristics of patients

	Confirmed maternal alcohol consumption (n=8)	Alcohol consumption denied or unknown (n=92)
Facial dysmorphology	1	6
FAS facial characteristics	1	3
Prematurity	2	10
Low birth weight	1	11
Failure to thrive	2	13
Microcephaly	1	3
Developmental delay	0	15
Other neurological findings*	0	5

*Irritability, abnormal tone, appetite dysregulation, hearing loss, craniofacial abnormalities

hol, only two had manifestations that could be associated with partial FAS (a diagnostic category of FASD), and none had the facial dysmorphology associated with in utero alcohol exposure. One child was premature and had a low birth weight adjusted for gestational age, while the second child had microcephaly and failure to thrive.

For children with no documented in utero exposure to alcohol, one child had all the manifestations of FAS including a flat upper lip, microcephaly, low birth weight and failure to thrive. A second child, who was examined at age 12 months, also had all the features of FAS including microcephaly, failure to thrive, developmental delay, and midline facial hypoplasia. As shown in Table 2, many children without alcohol exposure had clinical characteristics associated with FASD including characteristic facial features, low birth weight or failure to thrive, and neurological deficits including developmental delay and microcephaly. Nineteen children had more than one problem. Over two-thirds of the mothers (10/14) with known drug usage but without alcohol exposure had children with one or more of the following: dysmorphism, low birth weight, failure to thrive, developmental delay, microcephaly, and/or other neurological abnormalities. This is in contrast to maternal alcohol consumption with or without drug usage where most of the children were asymptomatic.

Table 3 is a summary of our findings regarding the diagnosis of FASD. As part of our analysis, we characterized children at risk for attaining a diagnosis of FASD and this included the six children with known maternal ethanol exposure but no clinical manifestations (at risk for ARND), and five children with two of the three criteria for FAS but without confirmed maternal ethanol exposure. Of the latter five, one patient was a 5 month old child with FAS dysmorphology and developmental delay without growth failure, and the other four were children with developmental delay/microcephaly and growth failure, but without recognizable facial features of FAS.

ARND = alcohol-related neurodevelopmental disorders

Table 3. Summary of findings

FAS /+ history of alcohol exposure	0
FAS /- history of alcohol exposure	2
Partial FAS	2
ARBD	0
ARND	0
Potential to develop FASD*	5
Alcohol exposure/no signs of FASD	6

* No history of ethanol exposure but two of three clinical characteristics (FAS dysmorphology, growth failure, neurologic abnormalities)

ARBD = alcohol-related birth defects, ARND = alcohol-related neurodevelopment disorders

DISCUSSION

Among the children evaluated in a national medical adoption unit, 4% of the children met the criteria for a diagnosis of FASD, and another 11% were highly likely to receive a diagnosis of FASD either because of known alcohol exposure so that any neurologic, psychological or social adjustment abnormality discovered subsequently would place them in the category of ARND, or because they had two of three characteristics of FAS and would then meet the definition for FAS without confirmed maternal alcohol exposure.

It is likely that we are underestimating the true incidence of FASD and the number of children at risk in our population. Having a history of maternal alcohol exposure significantly impacts on the diagnosis of FASD. Without a confirmed history of maternal alcohol exposure, it is impossible to diagnose partial FAS, ARBD, or ARND so that the only diagnostic entity obtainable is FAS, which requires typical facial dysmorphology, growth failure and neurological manifestations. In contrast, in the presence of a history of maternal alcohol consumption, a child can be classified as FASD even with one of these abnormalities (partial FAS) or can be classified as ARBD or ARND if he or she has birth defects related to ethanol or neurological or social dysfunction.

In our patient population we often did not have information pertaining to the mother. Even when there was information, we could not be sure of its veracity as the information was often obtained by Child Protective Services personnel, and the parent may not have disclosed that she had consumed alcohol so as not to prejudice the legal and social status of her infant. In fact, one of the children in our study is listed as at risk for FAS without a history of maternal alcohol consumption (no information was available). Subsequently, when we examined a younger sibling, it became clear that the mother was an alcoholic who drank during her pregnancies. Based on the new information, this child should be reclassified as having FAS, thereby increasing the proportion of children with FASD to 5%.

Another reason for the underestimation of FASD in our population is that most of the children seen in our clinic were under the age of 1 year. Since many children were subsequently adopted, they were not followed in our clinic. This placed limits on our ability to diagnose FASD as many of the syndromatic and cognitive features of FASD do not become apparent until after 1 year of life. Reviewing studies performed in other countries, most children with FASD were diagnosed later in life, often after years of follow-up [5-7].

The high rate of both FASD and the risk for developing FASD in our selected population is not surprising when compared with studies conducted in other countries. FASD features were found in more than half the Russian orphans residing in

ARBD = alcohol-related birth defects

baby homes in Murmansk, Russia [16]. Similar findings were found for adopted children from Eastern Europe who were followed for a long time. Previous studies have also shown that children with FASD are over-represented in foster care and adoption [17]. For example, in a study done in Washington State, 50% of the surveyed children with FASD or FAS had at least one adoptive parent and 15.4% had foster parents [18].

A limitation of the current study is its generalizability to the general Israeli population. Clearly, the patients in our group were high risk and the incidence of FASD would be expected to be much lower among the general population of newborns. However, it would be reasonable to assume that there are mothers who ingested alcohol and did not give their children up for adoption or have them removed from the home. There are also mothers who would be considered normative and may have consumed alcohol early in the pregnancy prior to becoming aware of their pregnancy. This is quite common in other countries, and in the USA at least 50% of pregnant women drank alcohol during the 3 months prior to pregnancy recognition, and 1 in 20 of these women drank at moderate to heavy rates [19].

Our study is also in line with other recent reports suggesting an increase in alcohol abuse among Israelis, especially teenagers and young adults. This is reflected in statistics regarding the increasing abuse of alcohol in the general population [9], alcohol levels in fatal casualties in motor vehicle accidents [20], and the number of children brought to the emergency room with alcohol poisoning [21].

This raises the question why the reported incidence of FASD is so low in Israel given the findings in our study. Taking a conservative estimate that the incidence or risk of FASD in the general population is 100 to 1000 times lower than the 15% seen in our study, it would be expected that between 22.5 and 225 children born per year would be at risk for FASD (annual birth rate in Israel is approximately 150,000). This is far higher than the number of children carrying a diagnosis of FASD listed in hospitals or health insurance funds [8]. It is also far below the rates reported for other countries (USA, South Africa, Italy, Sweden) where the incidence of FASD in the general population ranges from 5% to 7% [5].

One possibility is that the rate of FASD in Israel is extremely low except in very high risk populations such as those seen in our clinic. This could be due to low rates of alcohol consumption and low rates of alcohol abuse among the different constituents of the Israeli population. While it is true that there are higher rates of abstinence in the population as compared to European countries, a large-scale survey sponsored by the Ministry of Health showed that the lifetime prevalence rate of alcohol abuse or dependence is 4.3% and that 5% of the population drank alcoholic beverages three or more times a week, which is comparable to European countries [10]. Specific segments of the population are at greater risk, including young adults, males, and immigrants from the former Soviet Union. Other studies have also shown that ethanol abuse is a significant problem among immigrants from the former Soviet Union and Ethiopia [22,23], and that the frequency of intoxication and binge drinking was increasing [9]. Hence, a low prevalence of alcohol intake or dependence is not a reason for the lower reported rates.

It is also possible that despite the high prevalence of drinking in the general population, pregnant women are very careful to avoid drinking during pregnancy. Weiss and colleagues [24] found that among 2477 woman who had given birth at a single medical center during the years 1999–2000, only 1.13% admitted to consuming alcohol during pregnancy – mostly small amounts and infrequently. However, the authors were very skeptical of their findings as they did not concur with reported prevalence rates in other countries; they suggested that there was significant under-reporting because of the fear of stigmatization, denial, and/or the reluctance to share personal information. This was borne out by a more recent survey done in 2010 sponsored by the Israel Anti-Drug Authority, which found that among 3815 postpartum women in three Israeli hospitals 17.1% reported that they consumed alcohol during pregnancy and 0.8% admitted to binge drinking at least once during the last 3 months of their pregnancy [13].

A third possibility is that there are protective genes for FASD that are either associated with diminished alcohol consumption or reduced teratogenic effects of alcohol on the fetus. The alcohol dehydrogenase 1B genotype is related to the risk for alcoholism, and there is a greater prevalence (20%) of the protective allele among Jews. While it is true that younger individuals (< 33 years old) carrying the protective allele had lower alcohol consumption (mean number of drinks per occasion 2.6), in those without the protective allele the mean number of drinks was 6.2, which is considered risky and unsafe drinking and within the ranges known to be associated with FASD [25]. There are also genes that are protective of the fetus, but there is no increased prevalence of this gene among Jews.

The most likely reason for under-reporting is the lack of awareness of health care providers or their lack of effort to either solicit a history of maternal alcohol consumption or examine children for features of FASD. In their study [8], Senecky and co-authors interviewed geneticists and child development specialists throughout Israel. Fifty percent of the respondents felt that "tens" or even "hundreds" of children with a potential diagnosis of FASD had been missed. Among the respondents, approximately 60% reported that there was low or insufficient awareness of FASD among physicians in Israel.

The current study suggests that the number of children with FASD being diagnosed is only the tip of the iceberg. This is unfortunate as early intervention may minimize many of the cognitive, behavioral and social problems associated with FASD [4]. Identification of mothers with a history of ethanol

consumption and the appropriate follow-up by the social services may prevent future cases in the same family. Attributing antisocial behavior to FASD may facilitate more appropriate interventions and lower the rate of recidivism [3].

CONCLUSIONS

This study shows that there is a high rate of FASD and a risk for developing FASD in a selected population of adopted or foster children in Israel. The study is limited in that it observed the patients for a short time and at a very early stage of development. While direct extrapolation to the general population is not possible, this study can confirm previous studies in Israel suggesting that FASD is under-diagnosed. Since intervention is important and potentially beneficial, it is crucial to identify children with FASD or at risk for developing FASD. Steps to improve the diagnosis of FASD in Israel would include large-scale studies of the pediatric population to determine the true incidence of maternal alcohol consumption and FASD as well as interventions to enhance the awareness of health care personnel regarding the need to assess pregnant women for ethanol exposure and clinical manifestations of FASD among children and adults.

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"Neither a man nor a crowd nor a nation can be trusted to act humanely or to think sanely under the influence of a great fear"

Bertrand Russell (1872-1970), British philosopher, mathematician, author, and Nobel Prize laureate

"The memories of a man in his old age are the deeds of a man in his prime"

Pink Floyd, English rock band that achieved worldwide success with their progressive and psychedelic rock music. Their work is marked by the use of philosophical lyrics, sonic experimentation, innovative album art, and elaborate live shows

Compliance with Eye Care in Glaucoma Patients with Comorbid Depression

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ABSTRACT: **Background:** Comorbid depression may play an important role in non-compliance with medical treatment among patients with chronic illnesses. Glaucoma is a potentially blinding chronic disease requiring life-long commitment to medical therapy. Failure to adhere to anti-glaucoma treatment may lead to disease progression and visual loss.

Objectives: To assess the prevalence of depressive symptoms in glaucoma patients and the association between these symptoms and non-compliance with anti-glaucoma therapy.

Methods: In this cross-sectional observational study, compliance with pharmacotherapy was assessed with the Morisky Medication Adherence questionnaire (eight items). Screening for depression was performed by means of the CES-D (Center for Epidemiologic Studies Depression scale). The association between depression and compliance rates was analyzed.

Results: The study group comprised 76 glaucoma patients; 19.7% of the subjects were classified as non-compliant (Morisky cutoff < 10) and 21.1% suffered from depression (CES-D cutoff ≥ 16). We found a similar level of non-compliance when comparing depressed with non-depressed glaucoma patients. However, a correlation was observed between the level of depression and the level of non-compliance (P = 0.04).

Conclusions: Our study revealed a similar rate of depression in glaucoma patients and the general Israeli population. The presence of depression was not associated with the presence of non-compliance, yet the level of depression was associated with the level of non-compliance.

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KEY WORDS: glaucoma, compliance with glaucoma therapy, glaucoma medical therapy, depression

It is estimated that over 60 million people worldwide currently suffer from glaucoma, of whom 4.5 million are estimated to become legally blind [1]. In Israel glaucoma was documented to be the third cause of blindness, accounting for nearly one-eighth of the cases [2]. Despite significant progress in the diagnosis and treatment of glaucoma, a high percentage of patients still lose their sight. One pertinent contributing factor to this problem is low compliance with anti-glaucoma treatment [3].

Estimates of compliance with pharmacotherapy in patients with glaucoma vary, the highest compliance rate being 77% [4,5]. Nearly half the patients with glaucoma or suspected glaucoma stop refilling their drug prescriptions within half a year of treatment, and only 37% continue refilling their prescriptions after 3 years of treatment. The lack of compliance with glaucoma treatment is important because it often results in visual loss [3]. The main cause for reduced compliance is forgetfulness [6]. Other causes include improper application of eye care, adverse effects of the medication, inconvenient follow-up visits, disbelief in treatment benefit, and termination of eye care prior to refilling the prescription [6].

Depression is a common condition that may be concurrent with many chronic illnesses, such as hypertension, cancer, lung and heart diseases [7]. In chronic patients, comorbid depression has been associated with reduced compliance with medical therapy [8]. Depressed patients are three times more likely to be non-compliant with medical treatment than non-depressed [9]. Furthermore, antidepressant treatment was found to improve compliance in patients with human immunodeficiency virus [10].

The prevalence of depression among patients with eye morbidity varies greatly in different studies. In some studies glaucoma patients were found to have a high prevalence of depression [11,12], while others showed no such association [5,13]. The aim of the present study was to assess the prevalence of depressive symptoms in glaucoma patients and the possible association between depression and non-compliance with anti-glaucoma therapy.

PATIENTS AND METHODS

This study was approved by the Institutional Review Board of the Wolfson Medical Center and adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability

and Accountability Act regulations. Written informed consents was obtained from all participants.

The patients were recruited from the outpatient clinics at the Wolfson Medical Center and Bat Yamon Medical Center. The data were collected between June 2007 and March 2008. The inclusion criteria were diagnosis of glaucoma, age 18 years and above, and use of anti-glaucoma eye drops. Patients were excluded if they were unable to independently administer eye drops due to physical disability or cognitive impairment.

All subjects underwent comprehensive general and ophthalmic evaluation, including medical and ophthalmic history, best corrected visual acuity test, slit-lamp examination, intraocular pressure measurement, and Humphrey visual field test with Swedish interactive threshold algorithm (SITA) standard or FASTPAC 24-2 (Carl Zeiss Meditec, Dublin, CA, USA). All participants completed two questionnaires evaluating depression and compliance with glaucoma treatment.

Depression was evaluated by means of the Center for Epidemiologic Studies scale screening test (CES-D) [14]. It is a 20-item questionnaire with statements corresponding to characteristic symptoms of depression experienced during the week prior to completion of the scale. Every item is graded from 0 (experienced for less than 1 day) to 3 (experienced for 5–7 days). The final score ranges from 0 to 60. Scores of 16 and above are indicative of depression, while higher scores suggest a more severe depression. The CES-D has been well validated and widely used in research settings, including glaucoma studies [11,12,15].

Compliance was evaluated by means of the eight-item Morisky Medication Adherence Scale [16], which consists of seven yes/no questions, graded 1 or 0, respectively. The eighth question – "How often do you have difficulty remembering to take all your medications?" – is graded 1 (all the time) to 5 (never to rarely). Compliance scores range from 1 to 12. Scores of 9 and below indicate lack of compliance. The questionnaire was found to be reliable (alpha 0.83) and correlated with disease control and pharmacy refills [17,18].

The question "How often do you miss scheduled appointments?" was added to the questionnaire, adapted from the Hill-Bone Compliance to High Blood Pressure Therapy scale. It is scored 1 (never) to 4 (always) [19]. In order to assess the frequency of the medical follow-up the patients were asked an additional question: "How often do you meet your ophthalmologist?"

STATISTICAL ANALYSIS

Differences between groups were assessed with Student's *t*-test and the Mann-Whitney test. Exploratory analyses were conducted to recognize possible mediators or modifiers of compliance such as age, gender, marital status, place of birth, years of education, disease duration and severity, type and number of eye drops, medical comorbidity, visual acuity, intraocular pressure, and ophthalmic procedures. The Kolmogorov-Smirnov test was used to examine the distribution of the continuous variables of the study population. Bivariate analysis using chi-square correlations and Spearman's rho tested the association between depression and compliance. *P* value of 0.05 was selected as the threshold of statistical significance. In addition, a linear regression model was constructed to determine the relationship between the level of depression and the level of compliance, controlling for potential confounding variables listed above.

RESULTS

Seventy-six glaucoma patients participated in this cross-sectional analysis. The patients had suffered from glaucoma for an average of 7.26 years (range 0.1–45 years) and were treated with one type of eye drops per day (median, range 0–4).

COMPLIANCE WITH GLAUCOMA TREATMENT

The average Morisky score was 10.21 (SD 1.73). Sixty-one patients (80.3%) were classified as compliant (Morisky score ≥ 10) and 15 patients (19.7%) were non-compliant. The median Morisky score was 11 (range 10–12) among the compliant patients and 8 (range 2–9) in the non-compliant group. The median ophthalmic follow-up interval was 3 months (range 1–12 months). Only 13 patients (17.1%) reported that they had "sometimes" missed appointments.

- **General and glaucoma parameters and compliance**

There were no significant differences between the compliant and the non-compliant groups in any demographic characteristic [Table 1]. We observed that the visual field mean defect in the worse eye was statistically greater in the non-compliant patients. There were no significant differences between the compliant and non-compliant groups in visual acuities, intraocular pressure, ophthalmic procedures, type or number of drops of glaucoma eye treatment, better eye visual field parameters, or worse eye visual field pattern standard deviation [Table 2]. Compliance was marginally better when the treatment was identical in both eyes versus unilateral or different eyes (86.3% vs. 66.7% vs. 83.3% respectively, $P = 0.06$), although a linear result was not produced.

- **Compliance and reported medical follow-up**

A significant association was found between non-compliance and frequent medical follow-ups. Compliant patients met their ophthalmologist every 3 months (median, range 1–12), compared to every 2.5 months (median, range 1–12) for non-compliant patients ($P = 0.04$). No association was found between missing a scheduled ophthalmic appointment and the Morisky score.

Table 1. Compliance and patient demographics

	Non-compliant (N=15)	Compliant (N=61)	*P* value
Male/female ratio	5:10	35:26	0.09*
Age (mean ± SD)	71 ± 8.38	73.27 ± 11.28	0.46**
Marital status			0.4*
Married	9 (60%)	40 (65.6%)	
Divorced	2 (13.3%)	3 (4.9%)	
Widowed	3 (20%)	17 (27.9%)	
Single	1 (6.7%)	1 (1.6%)	
Country of origin			0.22*
Europe	4 (26.7%)	26 (42.6%)	
Balkan states	2 (13.3%)	10 (16.4)%	
Arab countries	9 (60%)	18 (29.5%)	
Caucasus	0	6 (9.8%)	
Latin America	0	1 (1.6%)	
Born in Israel	2 (13.3%)	12(19.7%)	0.5*
Years of residence in Israel (mean ± SD)	49.46 ± 15.38	47.45 ± 19.8	0.71**
Years of education (mean ± SD)	9.2 ± 4.12	10.72 ± 4.48	0.23**
Medical comorbidity	13 (86.7%)	53 (86.9%)	0.9*
Sedative/antidepressant/neuroleptic drugs	4 (26.7%)	7 (11.5%)	0.21*
Treatment in hospital clinic	5 (33.3%)	19 (31.1%)	0.87*

SD = standard deviation * Chi-square tests ** Independent-sample two-tailed *t*-test

Table 2. Compliance and glaucomatous characteristics

	Non-compliant	Compliant	*P* value
Glaucoma (yrs), median (min-max)	4 (0.2–15)	5 (0.1–45)	0.7§
Past ophthalmic procedures			0.34†
None	4 (26.7%)	26 (42.6%)	
Not related to glaucoma	6 (40%)	24 (39.3%)	
Glaucoma related	5 (33.3%)	11 (18%)	
No. of drops in the more treated eye, median (min-max)	1 (0–4)	1 (1–4)	0.5§
No. of drops in the less treated eye, median (min-max)	1 (0–3)	1 (1–3)	0.8§
Treatment in both eyes			0.06†
Identical	7 (46.7%)	44 (72.1%)	
Unilateral	6 (40%)	12 (19.7%)	
Different	1 (6.7%)	5 (8.2%)	
Untreated	1 (6.7%)	0	
Type of drops per patient			
Alpha-agonist	3 (20%)	16 (26.2%)	0.74†
Beta-blocker	11 (73.3%)	14 (77%)	0.74†
Carbonic anhydrase inhibitor	7 (46.7%)	35 (57.4%)	0.45†
Prostaglandin analog	8 (53.3%)	37 (60.7%)	0.6†
Visual acuity in better eye logMAR, median (min-max)	0.18 (0–0.48)	0.2 (0–2)	0.35§
Visual acuity in worse eye logMAR, median (min-max)	0.5 (0.1–3.4)	0.39 (0–3.4)	0.45§
IOP in lower pressure eye (mmHg), median (min-max)	13 (2-20)	13 (8-22)	0.27‡
IOP in higher pressure eye (mmHg) median (min-max)	16 (12-34)	15.5 (8-24)	0.16§
Visual field test			
MD in better eye (mean ± SD)	-5.5 ± 4.19	-4.89 ± 4.79	0.66‡
MD in worse eye (mean ± SD)	-12.23 ± 6.32	-8.63 ± 6.01	0.05‡*
PSD in better eye (mean ± SD)	2.5 ± 2.88	3.04 ± 3	0.55‡
PSD in worse eye (mean ± SD)	6.53 ± 4.2	5 ± 3.61	0.17‡

IOP = intraocular pressure, MD = mean defect, PSD = pattern standard deviation, SD = standard deviation, logMAR = logarithm of the minimum angle of resolution

* Statistically significant ‡ Independent-sample two-tailed *t*-test

† Chi-square tests § Mann-Whitney test

DEPRESSION

The average CES-D score was 8.65 (SD 9.11). Sixteen patients (21.1%) were defined as depressed (CES-D score ≥ 16) and 60 patients (78.9%) were not depressed. The median CES-D score was 3 (range 0–3) among the non-depressed patients as compared to 22.5 (range 16–34) among the depressed patients.

- **General and glaucoma parameters and depression**

There were no significant demographic differences between the depressed and non-depressed groups, except for the duration of residency in Israel, which was longer among the depressed than the non-depressed patients (55.43 vs. 45.83 years, $P = 0.02$). There were no differences in the use of general medications, including oral beta-blockers, sedatives, antidepressants or neuroleptic drugs.

There were no significant differences between the depressed and non-depressed groups regarding visual acuity, intraocular pressure, visual field test, ophthalmic procedures, or monotherapy. No differences in the rate of depression were found when comparing the type of eye drops, including topical beta-blockers.

- **Depression and compliance with treatment**

Among the 16 depressed patients 4 were non-compliant (25%), while among the 60 non-depressed patients 11 were non-compliant (18.3%). The median Morisky score was 10 (range 7–12) among the depressed patients and 11 (range 2–12) among the non-depressed. The median CES-D score was 5 (range 0–34) in the compliant group and 6 (range 0–33) in the non-compliant group. No significant association was demonstrated between the presence of non-compliance and the presence of depression ($P = 0.55$). However, a correlation was found between the quotient of depression (CES-D score) and the level of non-compliance (Morisky score, $r = -0.23$, $P = 0.04$).

DISCUSSION

The present study examined the prevalence and the severity of depressive symptoms in patients with chronic glaucoma, as well as the influence of comorbid depression on compliance with anti-glaucoma treatment. One-fifth (21.1%) of the studied patients were found to be depressed. One-fifth (19.7%) of the patients in this cohort were non-compliant with anti-glaucoma medication. The existence of depression was not associated with reduced compliance. However, a correlation was demonstrated between the severity of depression and the level of non-compliance, hence the more depressive symptoms the patient had, the lower his or her compliance.

Evaluating compliance to treatment is challenging. There are various assessment methods, such as blood or urine drug level measures, pill count, electronic monitoring devices, patient interviews and pharmacy records [5,8,15]. Unfortunately,

most of these tools are inaccurate. Moreover, there are even fewer available reliable techniques when assessing topical eye treatments. Patients' self-report was found to underestimate the true rate of non-compliance when compared with medication monitors [20].

Studies that examined the rate of compliance in glaucoma patients used patient response to a single question (omitting over 10% of the weekly dose, or missing more than two drug doses a week), prescription refill patterns from the health insurance fund database, or electronic monitoring devices [5,11,12,15]. In our study we chose to use a detailed questionnaire, the eight-item Morisky Medication Adherence Scale, which has proven to be a highly reliable screening tool [18]. This scale performed comparably to electronic devices, yet it was never validated in comparison to an eye-drop electronic monitoring device [21]. Further studies should explore compliance with treatment using self-reports as compared to electronic measurements.

Using a single-question assessment, Pappa et al. [12] found a depression rate of 46% and a compliance rate of 58% among 100 Greek patients, concluding that there was an association between depression and lack of compliance. Jayawant et al. [11] examined the compliance of elderly glaucoma patients according to prescription refill patterns and found that the rates of depression and compliance were 9.27% and 49%, respectively. The researchers reported a 29% decrease in compliance among depressed patients.

In the current study, we discovered a much higher compliance rate (80.3%) than the aforementioned publications. This rate is also similar to the upper estimates of compliance in glaucoma, ranging from 49% to 77% [4,11,12]. This might explain the reduced coexistence of non-compliance and depression among our patients. The wide estimate of compliance may result from population differences or diversity in compliance measurement methods, especially since self-report questionnaires might overestimate compliance.

Similar to our study, other glaucoma studies [5,15] that used electronic monitoring devices to measure compliance did not find an association between compliance and depression. Friedman et al. [15] reported a compliance rate of 55.6% in 196 patients who completed 8 weeks of follow-up (only 69% of the original cohort). A univariate analysis found a correlation of depression with non-compliance, while an adjusted analysis did not find such a connection. Hollo et al. [5] found a compliance rate of 77% and a mild to moderate depression rate of 12.1% with no correlation between the two conditions.

In the present study glaucoma severity was associated with decrease in compliance. This correlates with previous reports describing reduced compliance in progressive disease [5,12]. The reduced compliance might have accelerated the disease progression, yet negative causality is also applicable: patients with severe glaucoma might have technical difficulties in administering eye drops or feel desperate because of the alleged lack of treatment benefit and are consequently less compliant. The non-compliant patients had frequent medical follow-ups; this might also represent a more severe disease that required a frequent follow-up. It is known that an inadequate follow-up is a predictive factor for non-compliance [8]. No association was found between the duration of the glaucoma or missed appointments and the level of compliance, contradicting previous findings [8,12].

Different types of eye drops have been reported to yield varied compliance rates. Prostaglandins were suggested to have the highest compliance rate, as compared to beta-blockers, alpha-agonists, and carbonic anhydrase inhibitors [22]. Our study, similar to that of Pappa et al. [12], found no difference in compliance with treatment when comparing different types of eye drops. The use of different types of eye drops, including topical beta-blockers, had no influence on the rate of depression either, which was similar to previous reports.

The rate of depression among the study participants was 21.1%. This prevalence is similar to the reported rate of depressive symptoms in the general Israeli population, ranging from 14.3% to 24% [24,25]. Similarly, Wilson et al. [13] failed to observe an increase in depression rates among American glaucoma patients. Those authors reported depression rates of 7.4% and 13.3% in the glaucoma group and control group, respectively. Other studies reported depression rates of 12.1% and 9.27% among Hungarian and American glaucoma patients, respectively [5,11]. A Greek study found that 46% of 100 glaucoma patients were depressed, without comparison to a control group or to the general population [12].

In conclusion, lack of compliance was found to be associated with frequent follow-ups and a more severe decrease in visual field test results. The presence of depression was not found to be associated with the presence of non-compliance, yet the *level* of depression correlated with the *level* of non-compliance, or in other words, severely depressed patients tend to comply less with treatment. Therefore, caregivers should give special attention to this population group and provide them with the appropriate pharmaco-behavioral care required to increase their compliance.

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Capsule

Non-canonical inflammasome activation targets caspase-11

Caspase-1 activation by inflammasome scaffolds comprised of intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and the adaptor ASC is believed to be essential for production of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 during the innate immune response. Kayagaki and team show, with C57BL/6 Casp11 gene-targeted mice, that caspase-11 (also known as caspase-4) is critical for caspase-1 activation and IL-1β production in macrophages infected with *Escherichia coli*, *Citrobacter rodentium* or *Vibrio cholerae*. Strain 129 mice, like Casp11–/– mice, exhibited defects in IL-1β production and harbored a mutation in the Casp11 locus that attenuated caspase-11 expression. This finding is important because published targeting of the Casp1 gene was done using strain 129 embryonic stem cells. Casp1 and Casp11 are too close in the genome to be segregated by recombination; consequently, the published Casp1–/– mice lack both caspase-11 and caspase-1. Interestingly, Casp11–/– macrophages secreted IL-1β normally in response to ATP and monosodium urate, indicating that caspase-11 is engaged by a non-canonical inflammasome. Casp1–/–Casp11129mt/129mt macrophages expressing caspase-11 from a C57BL/6 bacterial artificial chromosome transgene failed to secrete IL-1β regardless of stimulus, confirming an essential role for caspase-1 in IL-1β production. Caspase-11 rather than caspase-1, however, was required for non-canonical inflammasome-triggered macrophage cell death, indicating that caspase-11 orchestrates both caspase-1-dependent and independent outputs. Caspase-1 activation by non-canonical stimuli required NLRP3 and ASC, but caspase-11 processing and cell death did not, implying that there is a distinct activator of caspase-11. Lastly, loss of caspase-11 rather than caspase-1 protected mice from a lethal dose of lipopolysaccharide. These data highlight a unique pro-inflammatory role for caspase-11 in the innate immune response to clinically significant bacterial infections.

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Eitan Israeli

The Clinical Significance of Ventricular Arrhythmias during an Exercise Test in Non-Competitive and Competitive Athletes

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ABSTRACT: **Background:** Sudden death in athletes can occur during sport activities and is presumably related to ventricular arrhythmias. There are no guidelines concerning athletes who develop ventricular arrhythmias during an exercise test. It is unclear whether they should be allowed to continue with their competitive activity or not.

Objectives: To investigate the long-term follow-up of athletes with ventricular arrhythmias during an exercise test.

Methods: From a database of 56,462 athletes we identified 192 athletes, less than 35 years old, who had ventricular arrhythmias during an exercise test. Ninety athletes had ≥ 3 ventricular premature beats (group A) and 102 athletes had ventricular couplets or non-sustained ventricular tachycardia during an exercise test (group B). A control group of 92 athletes without ventricular arrhythmias was randomly selected from the database (group C).

Results: All athletes, except one who died from a dilated cardiomyopathy, were alive during a follow-up period of 70 ± 25 months. An abnormal echocardiogram was obtained in seven athletes from group A (10%), four from group B (5%), and one from group C (3%) (not significant). An abnormal echocardiogram was more likely to be present in competitive athletes ($P = 0.001$) and in female athletes ($P = 0.01$).

Conclusions: Our results showed that ventricular arrhythmias during exercise are more commonly associated with cardiovascular abnormalities in young competitive athletes and in female athletes. When present, they necessitate a thorough investigation and follow-up.

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KEY WORDS: arrhythmias, exercise testing, echocardiography, competitive sports

Sudden death in young athletes has a major impact on the lay and medical communities. Identifying athletes at risk of sudden death remains a major challenge. Ventricular premature beats occur among athletes with the same frequency as in the general population but they usually disappear with exercise. In contrast, complex ventricular arrhythmias should prompt a search for underlying heart disease. In the majority of cases these arrhythmias are supposedly part of the "athlete's heart syndrome" and do not increase the risk of sudden death in athletes with an apparently normal heart [1-4]. The data available in the literature deal with ventricular arrhythmias assessed mainly by 24 hour ambulatory electrocardiograms [5,6].

To date, there are no guidelines concerning athletes who develop ventricular arrhythmias during an exercise test. The aim of this retrospective study was to look at the long-term follow-up of these athletes to determine whether the development of ventricular arrhythmias during an exercise test is an indicator for future cardiovascular events or cardiovascular abnormalities.

PATIENTS AND METHODS

PATIENT SELECTION

The athletes' records at the Wingate Institute of Sports for the period January 1995 to August 2007 were reviewed. Athletes were referred by different athletic organizations for preparticipation screening before engagement in sport activities. This was not part of a prospective organized national screening program for athletes.

According to the Israeli guidelines for sports medicine, all athletes should undergo physical examination before engaging in sports activities prior to each game season. Additionally, each athlete must undergo an exercise test at age 17, 23, 27, 32, 34 and yearly thereafter. Athletes with an abnormal physical examination, an abnormal electrocardiogram and/or an abnormal exercise test are referred for further workup as needed. All the records of clinical data on the athletes in our study were kept in a database maintained by the Wingate Institute. The exercise tests were retrospectively analyzed by an electrophysiologist for the purpose of this study. All VPB,

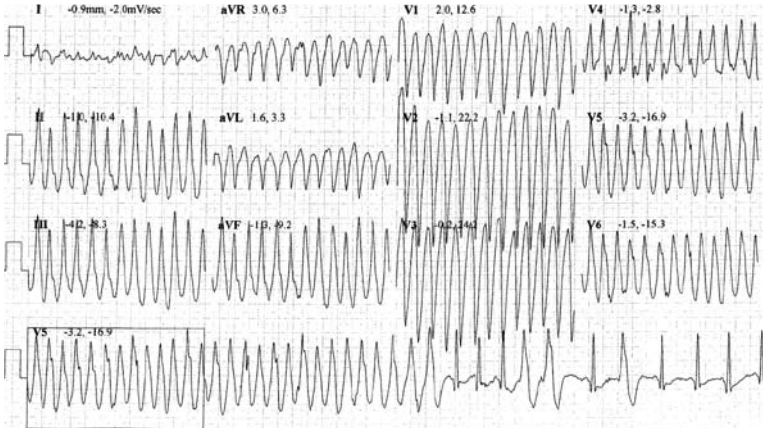
VPB = ventricular premature beats

Table 1. Classification of sports

	A: Low dynamic component	B: Moderate dynamic component	C: High dynamic component
High static component	Gymnastics, sailing, climbing, water skiing, weight lifting, windsurfing	Body building, wrestling	Boxing, canoeing, cycling, rowing, speed skating, triathlon
Moderate static component	Equestrian, motorcycling	Football, jumping, figure skating, rugby, running, surfing, swimming	Basketball, ice hockey, lacrosse, running (middle distance), swimming, handball
Low static component	Bowling, cricket, golf	Baseball, softball, fencing, table tennis, volleyball	Squash, running (long distance), soccer, tennis

Modified from the 36th Bethesda conference, task force 8 [8].

Figure 1. Ventricular arrhythmias during an exercise test in athletes.
Twelve-lead ECG at peak exercise shows a very rapid ventricular tachycardia at 300 beats/min, which subsided immediately during recovery from exercise. The athlete was asymptomatic. He was a competitive weight lifter and was disqualified from competitive sports. He underwent a thorough workup that included an echocardiogram and an MRI; both were normal.



ventricular couplets and runs of non-sustained ventricular tachycardia were counted from the tracings obtained during the exercise test and during the recovery period. (NSVT was defined as three or more consecutive ventricular beats). The athletes' baseline ECG were analyzed according to the most recent recommendations for interpretation of 12-lead ECG in the athletes [7]. Athletes with ventricular premature beats only at rest before exercise were excluded from the study. Athletes older than 35 were also excluded from the study in order to decrease the likelihood of enrolling athletes with coronary artery disease.

Each of the athletes completed a detailed questionnaire and underwent a thorough physical examination by a sports physician. All athletes underwent an exercise test adhering to the Astrand protocol (maximal exercise test with change of speed and steepness of the treadmill every minute). At the time of the exercise test no athlete was taking a beta-blocker or an anti-arrhythmic drug. Athletes who developed ventricular arrythmia during the exercise tests underwent a more thorough cardiovascular workup that included a Holter monitor (n=37), echocardiogram (n=182), magnetic resonance imaging (n=3) and electrophysiologic study (n=4).

An abnormal echocardiogram was defined as follows: valve abnormalities at least moderate in severity, hypertrophy defined as > 13 mm, and dilated cardiomyopathy diagnosed based on a dilated left ventricular cavity of ≥ 60 mm in end-diastole and a left ventricular ejection fraction < 45%. Athletes who had significant arrhythmias during exercise or recovery were disqualified from competitive sports until completion of the medical workup. Fifteen athletes from Group A and 22 athletes from Group B were disqualified from sports participation because of ventricular arrythmia during exercise.

The athletes were engaged in different sport disciplines. The different sport activities were divided into three groups: low (class A), moderate (class B) and high (class C), according to the task force for the classification of sports [8]. This classification was used to simplify the data analysis [Table 1].

The study was supported in part by a grant from a medical equipment company. The sponsors had no involvement in the study design, data analysis, or manuscript preparation. The authors are responsible for the accuracy and completeness of the data and the analyses.

STUDY DESIGN

We conducted a matched case-control study that compared the long-term outcomes in athletes who had VPB, ventricular couplets and NSVT during an exercise test with the outcomes in a similar group of athletes who had no ventricular arrythmias during an exercise test [Figure 1]. The study design was approved by the institutional review boards of the Wingate Institute and the Assaf Harofeh Medical Center. Written informed consent was waived for the athletes whose records were reviewed.

STATISTICAL ANALYSISS

Statistical analysis was performed using SPSS version 13 software. Data are expressed as mean ± standard deviation. Differences between means were assessed by a paired Student's *t*-test or one-way ANOVA. Differences between proportions were assessed with the Pearson chi-square test, the Fisher exact test, McNemar test or Wilcoxon signed ranks test as appropriate. A two-tailed P value < 0.05 was considered to indicate statistical significance. Logistic-regression analysis was used to analyze the effect of several variables (age, gender, number of ventricular arrythmias, sports class, ECG abnormalities, competitiveness) on the occurrence of ECG abnormalities.

NSVT = non-sustained ventricular tachycardia

RESULTS

A total of 56,462 athletes underwent an exercise test for preparticipation screening at the Wingate Sports Institute between January 1995 and August 2007. From this database we identified 192 athletes who were under the age of 35 and had ventricular arrythmia during an exercise test. Ninety athletes had three or more VPB (group A) and 102 athletes had VPB and ventricular couplets or NSVT (range 3–30 consecutive beats) during an exercise test (group B) [Figure 1]. The control group comprised 92 athletes who were randomly selected from the group of athletes who had no ventricular arrythmias during their exercise test (group C).

DEMOGRAPHIC CHARACTERISTICS

Group A included 70 males (78%), Group B 83 males (81%) and Group C 77 males (84%). The mean age during their initial assessment was 25 ± 5 years for Group A (range 12–35, median 25), 25 ± 5 years for Group B (range 15–34, median 25) and 24 ± 5 years for Group C (range 14–35, median 24) [Table 2]. The athletes were engaged in different sport disciplines, most commonly running (98 athletes), followed by gymnastics (n=41), basketball (n=25), swimming (n=19), soccer (n=18), cycling (n=13), tennis (n=9), judo (n=7), horse riding (n=4), and miscellaneous disciplines (surfing, boxing, ice skating, water ball, netball, handball, volleyball, triathlon, fencing and weight lifting). The competitive athletes in this cohort took part in national or international Olympic events (Group A 27% competitive, Group B 19% competitive, Group C 21% competitive) (not significant). The remaining athletes were non-competitive.

ELECTROCARDIOGRAM

Eight athletes (9%) from Group A, 11 (11%) from Group B and 6 (6.5%) from Group C had an abnormal ECG (not significant). The ECG abnormalities included complete right bundle branch block (1 in group A, 2 in group B), negative T waves (3 in group A, 4 in group B, 3 in group C), right axis deviation (4 in group A, 3 in group B, 3 in group C), ST segment depression (1 in group B), q waves in the inferior leads (1 in group B). None were significant.

HOLTER MONITORING

Eight athletes from Group A had a Holter monitor, which showed VPB in three subjects and supraventricular arrhythmia in two subjects. Twenty athletes from Group B had a Holter monitor, showing VPB in 6 subjects and atrial premature beats in 2. Ten athletes from group C had a Holter monitor, showing VPB in 2 subjects and atrial premature beats in 2.

ECHOCARDIOGRAM

Echocardiogram was normal in 170 (93%) athletes and abnormal in 12 (7%). Sixty-eight athletes from Group A had an echocardiogram, which was abnormal in 7 (10%) [Table 2]. In Group B 79 athletes had an echocardiogram and in 4 of them it was abnormal (5%). Thirty-five athletes from Group C had an echocardiogram and in 1 of them it was abnormal (3%) ($P = 0.27$).

Table 2. Baseline characteristics of the 284 athletes

	Group A (N=90)	Group B (N=102)	Group C (N=92)	*P* value
Mean age (yrs, mean ± SD)	25 ± 6	25 ± 5	24 ± 5	0.19
Male gender	78% (70)	81% (83)	84% (77)	0.59
Sports class				
A	6% (5)	7% (7)	3% (3)	
B	45% (38)	36% (35)	28% (25)	
C	49% (41)	57% (55)	69% (61)	0.10
Competitive	27% (24)	19% (19)	21% (18)	0.39
Hours of training per week	7.3 ± 5.4	7.8 ± 9.1	7.1 ± 4.4	0.80
VA ex+recovery*				
No arrythmias	0	0	100% (92)	
› 3 VPB	53% (58)	46% (47)	0	
› 20 VPB	47% (42)	16% (16)	0	
Couplets	0	75% (76)	0	
NSVT	0	26% (26)	0	0.001
Arrhythmia morphology				
None	0	0	100% (92)	
RBBB	46% (41)	57% (56)	0	
LBBB	54% (49)	43% (42)	0	0.14
Arrhythmia axis				
None	0	0	100% (92)	
Right	56% (49)	43% (40)	0	
Left	44% (38)	57% (53)	0	0.10
Peak heart rate (bpm)	190 ± 10	190 ± 8	192 ± 8	0.13
VT-CL (msec)	0	287 ± 72	0	
Coupling interval (msec)	335 ± 77	319 ± 67	0	0.13
Abnormal ECG	9% (8)	11% (11)	6.5% (6)	0.57
Abnormal echocardiogram	10% (7)	5% (4)	3% (1)	0.27

Values are given as percentages, with number in parentheses

Sports class A,B,C according to the Bethesda classification of sports

* Ventricular arrythmia during exercise and or recovery.

VT-CL = ventricular tachycardia cycle length, VPB = ventricular premature beat, NSVT = non-sustained ventricular tachycardia, RBBB = right bundle branch block, LBBB = left bundle branch block

There was no significant difference in age, sports class, hours of training, peak heart rate during exercise, arrhythmia morphology, and ECG abnormalities between the group of athletes with a normal echocardiogram and the group with an abnormal echocardiogram. There was no statistically significant difference in the amount of ventricular arrhythmia during exercise in the athletes with an abnormal echocardiogram compared to those with a normal echocardiogram ($P = 0.27$).

Logistic-regression analysis was used to analyze the effect of several variables (age, gender, amount of ventricular arrythmia, sports class, ECG abnormalities and competitiveness) on the occurrence of echocardiographic abnormalities. According to our data, athletes who were competitive were six times more likely to have an abnormal echocardiographic finding than those who were not non-competitive ($P = 0.001$) and female athletes were four times more likely than male athletes to have an abnormal echocardiographic finding ($P = 0.019$).

The structural cardiovascular abnormalities in the 284 athletes included mitral valve prolapse (4 in group A, 2 in group B, 1 in group C), myxoma (1 in group A), dilated aorta (1 in group A and 1 in group B), atrial septal defect (1 in group B), dilated cardiomyopathy (1 in group B), a small aneurysm in the right ventricular outflow tract (1 in group B), low right ventricular ejection fraction (1 in group A), and an enlarged left ventricle (1 in group A). Two athletes from group A underwent surgery (one for coarctation of the aorta and one for an atrial septal defect).

ELECTROPHYSIOLOGIC STUDIES

Four athletes from Group B underwent an electrophysiologic study in different local hospitals. The information on the protocol used is not available. Two athletes had a normal study, one athlete was inducible to atrial fibrillation, and one athlete was inducible to polymorphic ventricular tachycardia.

MAGNETIC RESONANCE IMAGING

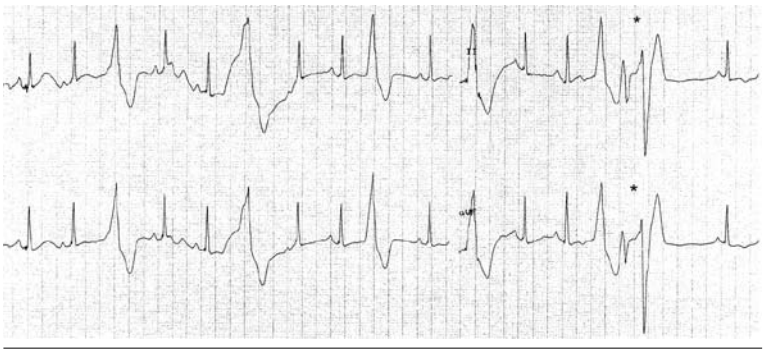
Three athletes from Group B had a cardiac MRI. The findings were normal in two athletes and one athlete had a small aneurysm in the right ventricular outflow tract.

FOLLOW-UP

The athletes were contacted by phone after a mean follow-up period of 70 ± 25 months. According to the Ministry of Interior database, the athletes who were lost to follow-up were alive in June 2009 except for one athlete from Group A, one athlete from Group B and 5 athletes from Group C about whom we could not obtain information.

One athlete from group B died during the follow-up period. He was 24 years old at his initial exercise test. This athlete played tennis and trained for about 7 hours weekly as a tennis coach and was not engaged in competitive sports. His exercise test showed frequent VPBs and ventricular couplets. The VPBs had a left bundle branch block and right axis morphology. The coupling interval was 300 msec and the cycle length of the ventricular couplets was 220 msec [Figure 2]. The VPB had a bizarre shape and were wide (QRS width 140–400 msec). His ECG showed deep negative T waves. The initial echocardiogram was normal and he was authorized to continue playing tennis. After 3 years he was diagnosed with a dilated cardiomyopathy and had an implantable defibrillator inserted. He subsequently underwent a left ventricular assist device implantation. He died one year after the diagnosis of dilated cardiomyopathy (3 years after the initial exercise test).

Figure 2. Rhythm strip of leads II, aVF, V5 during the initial exercise test. Note the wide and bizarre ventricular premature beats and the polymorphic non-sustained ventricular tachycardia of three consecutive beats as indicated by the asterisk.



DISCUSSION

Sudden death in young and healthy-appearing athletes is a rare event but its occurrence creates an immense impact on the lay and medical communities [9-13]. According to a study by Corrado et al. [14], sports activity in adolescents and young adults is associated with an approximately three times greater risk of sudden cardiovascular death. The same investigators showed that there was a decline in sudden cardiovascular death in young competitive athletes after the implementation of preparticipation cardiovascular screening in Italy [15].

The mechanism of ventricular arrythmia in athletes is unclear [16-18]. Some arrhythmias may be secondary to the high sympathetic tone during exercise and others may be secondary to structural heart disease. Heidbuchel and coauthors [19] studied the role of an electrophysiologic study for risk stratification in athletes with ventricular arrythmia, with induction of sustained ventricular tachycardia or ventricular fibrillation and a re-entry mechanism implicating a worse prognosis [19].

Since sudden death occurs in most cases during sports activities, what is the significance of ventricular arrythmia during a preparticipation screening exercise test in an athlete? Clinicians are faced with the dilemma of either considering these arrhythmias as a benign finding that is part of the 'athlete's heart' or considering them as potentially life threatening. A similar dilemma arises when faced with an abnormal ECG in an athlete. Some of these ECG changes may be part of the 'athlete's heart' and some may represent the initial expression of a cardiovascular disease [20].

In the present study, ventricular arrythmia during an exercise test occurred in 11% of the athletes (192/1712) who were less than 35 years old. Our data are similar to the findings of Biffi et al. [5,6] who studied the long-term significance of ventricular arrythmia in 24 hour Holter monitoring in trained athletes. According to their study, ventricular arrythmias are common in trained athletes and are usually not associated with underlying cardiovascular abnormalities. When ventricular arrythmias are associated with cardiovascular abnormalities, disqualification from competitive sports is recommended.

Another interesting finding in our study was the higher likelihood of an abnormal echocardiogram in female athletes and in competitive athletes with ventricular arrhythmias dur-

ing exercise. The high incidence in female athletes is probably related to the higher occurrence of mitral valve prolapse in women, whereas it can be speculated that the higher incidence of echocardiographic abnormalities in competitive athletes may be secondary to cardiac anatomical changes as a result of very strenuous physical activity.

The athletes with the more severe forms of ventricular arrythmia were automatically disqualified from competitive athletic activities and therefore we do not know if disqualification reduced the incidence of cardiovascular events in these athletes. Except for one athlete who died from a dilated cardiomyopathy, all athletes were alive and were doing well during the follow-up period. On the basis of our data it seems reasonable that in the setting of a large-scale preparticipation screening program, the occurrence of ventricular arrythmia during an exercise test may be useful in identifying athletes with structural heart disease or athletes at risk for the development of heart disease.

In a recent publication Sofi and collaborators [21] studied 30,065 athletes who underwent an exercise test before participation in competitive sports. They found that age > 30 years was significantly associated with an increased risk of being disqualified for cardiac findings during exercise testing. They also found that exercise testing can show pathological findings in athletes with innocent findings at physical examination and resting ECG [21]. A discussion on the cost-effectiveness of such an approach and its application worldwide is beyond the scope of this article [22]. Nevertheless, the findings of the present study provide useful information for the cardiology consultant faced with the challenging management of an athlete with ventricular arrythmia in countries that mandate exercise testing before engaging in competitive sports.

Our study has several limitations due to the fact that a small percentage of athletes were engaged in competitive sports. Additionally, not all athletes had an echocardiogram and athletes with ventricular arrythmia were more likely to undergo a workup. It is possible that disqualification from intensive training and competition could have favorably influenced the outcome and prevented cardiac events in athletes with severe forms of ventricular arrythmia. Therefore, the risk of engaging in competitive sports with such arrhythmias cannot be assessed from our data.

In conclusion, we investigated the clinical significance of ventricular arrythmia occurring during an exercise test in non-competitive athletes and competitive athletes. Ventricular arrythmia during exercise is more commonly associated with cardiovascular abnormalities in young competitive athletes and in female athletes. When present, they necessitate a thorough investigation and follow-up.

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Compliance of Hospital Staff with Guidelines for the Active Surveillance of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and its Impact on Rates of Nosocomial MRSA Bacteremia

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ABSTRACT: **Background:** The compliance of hospital staff with guidelines for the active surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) in Israel has not been determined.

Objectives: To evaluate the compliance of hospital staff with guidelines for the active surveillance of MRSA and assess its impact on the incidence of nosocomial MRSA bacteremia.

Methods: We assessed compliance with MRSA surveillance guidelines by assessing adherence to the screening protocol and reviewing medical and nursing charts of patients colonized with MRSA, and observed hand hygiene opportunities among health care workers and colonized patients. Rates of nosocomial MRSA bacteremia and of adherence with hand hygiene among overall hospital staff were obtained from archived data for the period 2001–2010.

Results: Only 32.4% of eligible patients were screened for MRSA carriage on admission, and 69.9% of MRSA carriers did not receive any eradication treatment. The mean rate of adherence to glove use among nurses and doctors was 69% and 31% respectively ($P < 0.01$) and to hand hygiene 59% and 41% respectively ($P < 0.01$). The hospital overall rate of adherence to hand hygiene increased from 42.3% in 2005 to 68.1% in 2010. Rates of nosocomial MRSA bacteremia decreased by 79.2%, from 0.48 (in 2001) to 0.1 (in 2010) per 1000 admissions ($P < 0.001$).

Conclusions: The compliance of medical and nursing staff with guidelines for active MRSA surveillance was poor. The encouraging increase in adherence to hand hygiene and concomitant decrease in nosocomial MRSA bacteremia is gratifying. The deficiencies in compliance with MRSA infection control policy warrant an adjusted strategy based on the hospital resources.

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KEY WORDS: methicillin-resistant *Staphylococcus aureus* (MRSA), nosocomial infections, medical staff, nursing staff, surveillance, hand hygiene compliance

Methicillin-resistant *Staphylococcus aureus* is a major cause of hospital-acquired infections in many countries around the world [1,2] and in recent years it has become a significant cause of community-acquired infections [3]. A recent study from Israel addressed the role of community acquired-MRSA in pediatric soft tissue infections [4].

In the last decade numerous reviews and consensus statements have endorsed policies to control the spread of nosocomial MRSA infections [5,6]. The most widely addressed policy is perhaps active MRSA surveillance to detect and isolate MRSA carriers among hospital admissions, mainly a subgroup of patients at high risk for MRSA carriage [7,8]. Nevertheless, there is still controversy regarding the role of active surveillance in the control of MRSA infections, with a few systematic reviews suggesting that there are no good data to support this approach [9,10].

In Israel, methicillin resistance among *S. aureus* isolates is variable. Some authors reported very low rates (~0.6%) of nasal MRSA carriage among healthy children and 7.6% among chronically institutionalized children [11], while others presented rates ranging from 11.4% in community-acquired infections, 27% among hospital-acquired infections, and up to 50% among patients in long-term care facilities [12]. The use of active MRSA surveillance to control nosocomial MRSA infections has been officially adopted by only a few hospitals in Israel. One group published an initial report describing its efficacy [13] and recently published a second report after 5 years of follow-up, proving that rates of nosocomial MRSA bacteremia can be reduced when using active surveillance together with contact isolation and monitoring to ensure compliance with screening and contact isolation guidelines [14].

Our medical center adopted a policy of active MRSA surveillance in 2005, restricted to a subgroup of patients at high risk for MRSA carriage. It is unclear to what extent the medical

MRSA = methicillin-resistant *Staphylococcus aureus*

and nursing staff complied with MRSA surveillance guidelines for detecting colonized patients, isolation procedures, and what treatment, if any, was prescribed to colonized patients.

The purpose of the present study was to assess the compliance of medical and nursing staff with active MRSA surveillance guidelines, assess adherence of hospital staff to hand hygiene, and investigate its impact on rates of nosocomial MRSA bacteremia.

PATIENTS AND METHODS

From 2005 and based on guidelines of the Society for Healthcare Epidemiology of America [8], the infection control unit at HaEmek Medical Center adopted a policy that all patients from the following categories should be screened on admission for MRSA carriage: a) patients with known history of MRSA colonization or infection, b) patients admitted from other hospitals/nursing homes, c) patients receiving long-term hemodialysis, d) patients readmitted to the hospital within 3 months from their discharge, e) patients who underwent a major operative procedure in the previous month, f) all patients admitted to the intensive care unit, and g) patients with pressure sores or permanent tracheostomy living at home. Samples were obtained for culture from anterior nares and from the perineum (only in bedridden patients). A sputum culture was obtained from intubated patients, and the skin was sampled in patients with dermatitis or open wounds. Due to the shortage of single rooms, no request was made to transfer positive carriers to single rooms or to put them with each other in the same room. Nosocomial MRSA bacteremia was defined as an MRSA-positive blood culture result from blood that was drawn 48 hours after admission. The local ethics committee at HaEmek Medical Center approved the study.

STUDY DESIGN

The study consisted of two parts – retrospective and prospective. In the retrospective part we assessed adherence to guidelines for active MRSA surveillance by reviewing medical and nursing charts of patients who were admitted from January 2006 to January 2007 and were found to be colonized with MRSA. In the prospective part we started by assessing the adherence of nursing staff to the screening protocol (the number of patients eventually screened from among all the patients who should have been screened according to the guidelines); this was carried out in four medical and two surgical wards for 2 successive months, 10 months apart (early 2006 and late 2006), and a third survey carried out in mid-2009. On identifying a colonized patient, we assessed the opportunities for hand hygiene and adherence of staff to contact isolation alerts by direct observations that were carried out during morning shifts and in different sections of the medical and surgical wards.

Adherence to hand hygiene practices was evaluated by direct observation of contacts between health care workers and patients during hand hygiene opportunities. Measuring hand hygiene adherence was evaluated in accordance with published guidelines [15]. We also monitored the thoroughness of cleansing and whether gloves were used and changed when indicated. The adherence rate was calculated as previously described [16]: the total number of acts of hand hygiene when the opportunity existed divided by the total number of hand hygiene opportunities.

MEASURING ADHERENCE OF HOSPITAL STAFF TO HAND HYGIENE (2005–2010)

The assessment of adherence to hand hygiene practices among hospital staff was performed as part of an ongoing hospital-wide campaign initiated by the hospital infection control unit in 2004 to increase awareness to hand hygiene practices among hospital staff (N. Titler, unpublished data). Data of adherence rates were archived in the division of quality control and were retrieved for the purposes of this study.

MICROBIOLOGIC ANALYSIS

Swabs were collected from the anterior nares and rectum of the patients and streaked on chromogenic agar (Hylabs, Israel). Plates were incubated overnight at 37°C, colonies with typical morphology were confirmed as *S. aureus* using Pastorex coagulase test (BioRad, Marnes-La-Co-quette, France) and for DNase production on DNase agar (HyLabs). Methicillin resistance was determined using the Kirby-Bauer disk diffusion test with a 30 µg cefoxitin disk. An inhibition zone diameter of ≥ 22 mm was considered diagnostic for methicillin-susceptible *Staphylococcus aureus*. Susceptibility testing for mupirocin was performed for MRSA eradication purposes using a 200 µg mupirocin disk on a Mueller-Hinton agar plate. Any zone was considered susceptible.

STATISTICAL ANALYSIS

We used the chi-square test to assess differences in compliance and adherence rates between the study groups. Data analysis was conducted using SPSS version 17 (SPSS Inc., Chigaco, IL, USA). Significance was determined at the 0.05 level.

RESULTS

RETROSPECTIVE PHASE

During the study period from January 2006 to January 2007 we obtained 2857 swabs (nares, perineum, and trachea) from 1321 adult patients; 103 swabs (7.8%) were positive for MRSA. Approximately two-thirds of the swabs were obtained in the medical wards. There was no documentation of a management MRSA plan in 69.9% of the medical charts. In only 29% of the patients was the eradication treatment started

within 24 hours of confirmation of the result. Among the patients who received eradication treatment, 78% had a daily application of nasal mupirocin ointment to the anterior nares for 5 days, 68% had their bed linen changed daily for 5 days, and only 4% had a daily bath with 4% chlorhexidine for 5 days. Among MRSA carriers, sticky "contact isolation" labels were found in 93% of the medical and nursing charts, and in 85% of cases a sticky "contact isolation" label was found on the patient's bed.

Among 103 patients who were found to be MRSA carriers during the study period, only 30.1% (n=31) received eradication treatment. The rest of the carriers did not receive any treatment during their hospitalization and no mention of MRSA carrier status was recorded in the discharge notes. During 1 year of follow-up, 34 colonized patients (33%) were readmitted, of whom only 6 were found to still be MRSA carriers. None of the patients who were identified as MRSA carriers was admitted within the following year with invasive MRSA infection.

PROSPECTIVE PHASE

Compliance with the MRSA surveillance protocol was initially assessed by determining the adherence rate to the screening protocol (number of patients screened from all patients who should have been screened). The adherence rate varied between the different departments, ranging from 17.3% to 61.5% in the medical departments and from 32.2 to 41.4% in the surgical departments [Table 1]. Overall, only 32.4% of the patients who should have been screened upon admission were eventually screened. Sixty-eight patients colonized with MRSA were identified during this part of the study, and only 42% of them received eradication treatment.

During this phase of the study we monitored 839 contact opportunities between health care personnel and colonized patients. The mean rate of adherence among nurses and doctors to glove use was 69% and 31% respectively ($P < 0.01$), to use of medical aprons 39% and 7% respectively ($P < 0.01$), and to hand hygiene 59% and 41% respectively ($P < 0.01$).

Table 1. Adherence rates to MRSA screening protocol at the departments of medicine and surgery

Department	No. of patients who should have been screened*	No. of patients actually screened	Adherence rate (%)
Medicine A	165	59	36.2
Medicine B	148	34	22.8
Medicine C	172	30	17.3
Medicine D	187	115	61.5
Surgery A	91	29	32.2
Surgery B	85	35	41.4
Total	848	275	32.4

ADHERENCE OF HOSPITAL STAFF TO HAND HYGIENE (2005-2010)

Adherence to hand hygiene was evaluated by direct observation between health care personnel and patients during hand hygiene opportunities. Figure 1 shows adherence rates among doctors, nurses and paramedical staff during 5 years of unscheduled yearly observations carried out by the division of quality control. In 2005, the hospital overall adherence rate for hand hygiene was 42.3% (40.1%, 52.2% and 41.2%, for doctors, nurses and paramedical staff, respectively), and in 2010 the overall adherence rate was 68.1% (58.5%, 73.3% and 56.6%, for doctors, nurses and paramedical staff, respectively) ($P < 0.01$ for each of the three groups).

RATES OF NOSOCOMIAL MRSA BACTEREMIA (2001–2010)

Figure 2 shows the rates of nosocomial MRSA bacteremia during the last decade. Rates of nosocomial MRSA bacteremia decreased by 79.2% from 0.48 cases for 1000 admissions (2001) to 0.1 cases for 1000 admissions (2010) ($P < 0.001$).

Figure 1. Adherence rates to hand hygiene among doctors, nurses and paramedical staff during 2005–2010

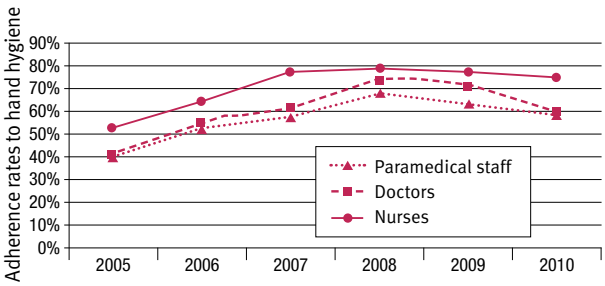
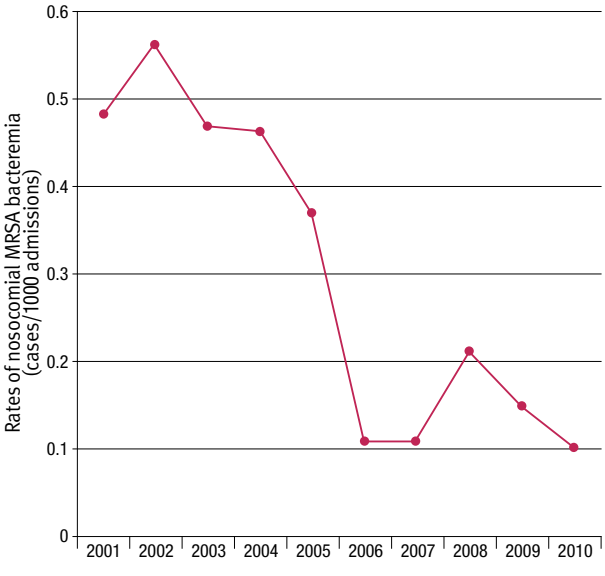


Figure 2. Rates of nosocomial MRSA bacteremia, 2001–2010



DISCUSSION

We found that the compliance of medical and nursing staff with active MRSA surveillance guidelines was poor. Overall, nearly two-thirds of patients who should have been screened for MRSA carriage on admission were not screened, and more than two-thirds of those who were found to be MRSA carriers did not receive any eradication treatment. Yet, and despite the poor compliance with active MRSA surveillance guidelines, rates of nosocomial MRSA bacteremia decreased continuously during the years 2005–2010.

The low compliance with MRSA surveillance guidelines in our hospital is rather disappointing but not surprising. Previous, and rather few, studies that assessed compliance rates among medical and nursing staff with active MRSA surveillance guidelines reported similar findings [17,18]. Furthermore, compared with nurses, doctors have performed poorly in all aspects of infection control precautions.

The role of policies and practices for the prevention, decolonization and eradication of MRSA in hospitals is widely accepted among health care providers; some have showed impressive results in reducing the MRSA disease burden [13,19-21], while others were more skeptical regarding its efficacy [10,22]. In support of those arguing against active surveillance and detection for MRSA, some hospitals reported significant reductions in the MRSA disease burden by the use of approaches that do not include an active "search and destroy" policy for MRSA [23-25]. The major interventions employed in these reports were mainly high adherence to hand hygiene. In our case and apart from the debate whether active surveillance for MRSA is effective in reducing the MRSA disease burden, its cost-effectiveness should be addressed, especially when nearly two-thirds of the colonized patients were in fact ignored with regard to their carrier state. The finding that rates of nosocomial MRSA bacteremia declined significantly over the last 5 years was obviously believed by policy makers to be secondary to active MRSA surveillance. However, based on our findings, it is reasonable to speculate that the observed reduction in nosocomial MRSA bacteremia was not related only to active MRSA surveillance, particularly in view of the impressive increase (~50%) in adherence to hand hygiene among hospital staff and a steady background of MRSA colonization rate among hospital admissions that has not changed during the past 3 years (2008–2010) (N. Bisharat, unpublished data). In our view, the poor performance in implementing acceptable infection control precautions, the significant deficiencies in contact isolation (lack of single rooms, low adherence to use of gloves, aprons, and hand hygiene) and afterwards, in providing eradication treatment for the colonized patients, argues against a major role for active MRSA surveillance, as a consolidated plan, in reducing rates of nosocomial MRSA bacteremia in our hospital.

Our study has a few limitations. The observers made every effort to be unobtrusive, but observation bias must be considered. In addition, our team of observers was a different team from the team that performed the yearly assessment of adherence to hand hygiene among the hospital staff, and therefore we cannot rule out observer-related variation. Second, our observations were made during morning shifts and included four medical and two surgical departments, which comprise almost 220 of the hospital's 500 beds; whether the results seen in these wards can be generalized to all the departments is uncertain. Third, it would have been ideal had we used a before-and-after study design, such as before and after implementing active MRSA surveillance with and without intervention in hand hygiene practices, but this is almost impossible since adherence to hand hygiene practices is perhaps the most important factor in precautions for controlling the spread of MRSA. Furthermore, our study was carried out at three time points (twice in 2007 and once in 2009), and it is therefore difficult to draw clear and definitive conclusions on the lack of efficacy of active MRSA surveillance.

In conclusion, our study highlights the major deficiencies in compliance with an MRSA infection control policy in our hospital. Nevertheless, this has not adversely affected the MRSA disease burden which has significantly declined over the past 5 years. Given its high costs and the poor compliance among hospital staff, it seems that there is a need to reconsider its formation. It would be more beneficial and cost-effective to focus on improving the rate of adherence to hand hygiene among hospital staff within a bundle of other interventions aimed to reduce hospital-acquired infections.

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Capsule

Desperately seeking XMRV – probably no connection to chronic fatigue syndrome

A report that patients with chronic fatigue syndrome (CFS) are infected with a retrovirus called XMRV attracted considerable attention, but follow-up work by other investigators failed to confirm the finding. In a study by Simmons and collaborators, nine laboratories – including the authors of the original report – independently analyzed blind-coded blood samples from 15 individuals previously found to be positive for the virus (14 with CFS) and 15 healthy controls previously found to be negative. Only the two laboratories associated with the original report detected XMRV. However, in these laboratories, the virus was found in healthy controls as often as in CFS patients and replicate samples yielded inconsistent results. In addition to showing that current assays for detecting XMRV are unreliable, these data support previous studies that questioned the association between XMRV and CFS.

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Eitan Israeli

Capsule

Awareness and attention

There has been a long-standing controversy on whether activity in the primary visual cortex is necessary for perceptual awareness. In human brain-imaging experiments, Watanabe et al. were able to dissociate perceptual awareness from simple attention. Awareness was manipulated in a binocular flash suppression paradigm, and attention was manipulated by using standard attentional instructions. Activity in the primary visual cortex varied little, whether a target was visible or not. However, activity in the human primary visual cortex varied when subjects attended to the target or ignored it. Thus, humans do not need primary visual cortical activity in order to be aware of seeing something.

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Eitan Israeli

Clinical, Electrophysiologic and Pathologic Findings in 10 Patients with Myotonic Dystrophy 2

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ABSTRACT: **Background:** Myotonic dystrophy type 2 (DM2) is an autosomal dominant, multisystem disorder caused by a CCTG tetranucleotide repeat expansion located in intron 1 of the zinc finger protein 9 gene (*ZNF9* gene) on chromosome 3q 21.3.
Objectives: To describe the clinical, electrophysiologic and pathologic findings in patients with myotonic dystrophy 2.
Methods: We evaluated 10 patients genetically, clinically and electrophysiologically during the years 2007 to 2008.
Results: All patients were of Jewish European ancestry. Among affected individuals, eight patients had symptoms of proximal muscle weakness, two had muscle pain, and two exhibited myotonia. On physical examination six patients had severe weakness of hip flexor muscles. Seven individuals underwent cataract surgery, and cardiac involvement was seen in one case. On the initial electromyographic (EMG) examination five patients demonstrated myotonic discharges; repeated studies showed these discharges in nine cases. Six muscle biopsies showed non-specific pathological changes. Seven patients had an affected first-degree relative with either a diagnosed or an undiagnosed muscular disorder consistent with an autosomal dominant trait.
Conclusions: DM2 may often present with proximal muscle weakness without myotonia. EMG may initially fail to show myotonic discharges, but these discharges may eventually show in most cases on repeated EMG. Thus, DM2 may be underdiagnosed and should be included in the differential diagnosis of adult patients of Jewish European ancestry presenting with proximal lower limb weakness.

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KEY WORDS: myotonia, myopathy, myotonic dystrophy type 2 (DM2), electromyography, muscle biopsy

Myotonic dystrophy type 2 was first recognized clinically in 1994 as a milder form of myotonic dystrophy known as DM1 [1]. DM2 was initially named proximal myotonic myopathy [1] or proximal myotonic dystrophy [2] until the genetic basis for this disorder was established in 2001, distinguishing this condition as a separate entity [3]. DM2 is an autosomal dominant, multisystem disorder partially resembling adult-onset DM1 [4]. It is characterized by skeletal muscle weakness and myotonia, cataracts, cardiac conduction abnormalities, and other systemic manifestations [1-4]. The underlying genetic mutation is a CCTG tetranucleotide repeat expansion located in intron 1 of the zinc finger protein 9 gene (*ZNF9* gene) on chromosome 3q 21.3 [5]. In normal alleles there are 11 to 26 tetranucleotide repeats, while in pathogenic alleles the number of repeats ranges from 75 to more than 11,000 (mean 5000 repeats) [6]. The disease has been described only in European patients and it is common in Germany and Finland. It has not yet been described in Israeli Jews.

DM2 = myotonic dystrophy type 2

PATIENTS AND METHODS

During the years 2007–2008 we reevaluated our patients with myopathy of unclear origin. DNA was obtained from 18 patients with a possible clinical diagnosis of DM2. The diagnosis of DM2 was established by genetic studies in 10 patients. The clinical, genetic and electrophysiological data from those 10 patients are reported here. The clinical data were taken from the files in the outpatient neuromuscular clinic and from the patients' reports.

Electrophysiological assessment was performed with the portable Key Point Medtronic EMG machine. Slit-lamp examination of all patients was performed at the ophthalmology clinic. Muscle biopsies were obtained from six patients. The specimens were quick frozen, sectioned, and stained with hematoxylin and eosin, Gömöri trichrome, periodic acid-Schiff, oil red O, NADH-TR, and ATPase at pH 9.4. Blood was drawn for DNA extraction after the patient signed an informed consent. Complete blood chemistry, blood cell count and serology were also studied.

GENETIC STUDIES

DNA samples were analyzed at the neuromuscular diagnostic center of the neurology department, University of Tampere

in Finland. Reverse transcriptase-polymerase chain reaction (repeat prime) was applied to amplify the four bases (CCTG) repeat region located in the first intron of the *ZNF9* gene [5]. The applied RP-PCR methods distinguish mutated expansion from normal DNA region by a pattern of differently sized fragments amplified [6]. Results were analyzed using fragment analysis with Gene-Mapper Software.

RESULTS

- **DM2 patients and families:** We identified 10 DM2 patients (7 were women). Seven individuals had an affected first-degree relative with an either diagnosed or undiagnosed muscular disorder, consistent with autosomal dominant trait. All patients were of European descent [Table 1]. Their age at diagnosis ranged from 31 to 79 years (mean age 65.4 years).
- **Age at onset and initial symptoms:** Patients reported having experienced the first symptoms of the disease from age 27 to 70 (mean age 52.8 years). The time from onset of symptoms to diagnosis ranged from 2 to 17 years (mean 7.5 years). Muscle symptoms (weakness, pain, myotonia) were the most common symptoms reported. Eight patients had symptoms of proximal lower limb weakness, such as difficulties running or walking long distances, rising from a sitting position, squatting or climbing stairs. Two individuals reported muscle pain and two patients described myotonia [Table 2].

RP-PCR = repeat primed polymerase chain reaction

Table 1. Age at diagnosis, descent and family member affected

Patient	Age (yrs)	Gender	Descent	Family member affected
1	77	Female	Romania	Sister
2	79	Female	Romania	Sister
3	31	Female	Poland	Mother
4	57	Female	Poland	Daughter
5	73	Male	Poland	–
6	68	Female	Bulgaria	–
7	77	Male	Germany	Brother
8	60	Female	Romania	-
9	67	Female	Russia	Father, sister
10	65	Male	Russia	Father, sister, uncle

Table 2. Initial symptoms

Initial symptoms	No. of subjects reporting each initial symptom
Myotonia	2
Weakness	8
Muscle pain	2

- **Distribution of muscle weakness:** All 10 patients exhibited proximal upper and lower limb muscle weakness. The iliopsoas muscle was the most severely affected. Other commonly affected muscle groups were the facial, neck flexors, and distal hand muscles [Table 3].
- **Muscle biopsy:** Muscle biopsies from the vastus lateralis and the deltoid muscles were obtained in six patients. Two specimens showed internal nuclei and nuclear clamps. Necrotic fibers, fibrosis with abnormal fiber type distribution, lobulated fibers, and non-specific myopathic changes were noted in one case each [Table 4].
- **Cataract:** Seven patients underwent cataract surgery extraction.
- **Electromyography:** EMG was obtained in 9 of the 10 cases. In five, the first EMG examination demonstrated myotonic discharges, while in four patients it showed either myopathic changes or neurogenic changes without myotonic discharges. On repeated EMG examination nine patients demonstrated myotonic discharges [Table 4].
- **Other manifestations:** Atrial fibrillation and hearing loss was reported by one patient aged 65.

EMG = electromyography

Table 3. Affected muscles

Muscle group	Muscle strength*	No. of subjects affected
Iliopsoas	0–2/5	6
	4/5	4
Facial muscle (orbicularis oculi)	5–/5	7
Neck flexors	4/5	5
Deltoid	4–4+/5	10
Palmar interossei	4/5	4

*Muscle strength was evaluated using the medical research council (MRC) scale

Table 4. Additional laboratory findings

Muscle biopsy results	No. of subjects affected
Fat only	1
Necrotic fibers, fibrosis, abnormal fiber type distribution	1
Lobulated fibers	1
Non-specific myopathic changes	1
Internal nuclei, nuclear clamps	2
EMG results	
1st examination	
Myotonia	5
Myopathy	1
Myopathy + muscle fibrillation	1
Myopathy + neuropathy	1
Neuropathy	1
2nd examination	
Myotonia	9

- **Serum creatine kinase levels:** Creatine kinase levels were mildly to moderately elevated in all patients, ranging from 500 to 1200 IU/L.

DISCUSSION

DM2 shares many clinical features with DM1, such as progressive muscle weakness, myotonia, cardiac arrhythmia, cataracts and other systemic manifestations. DM2 patients may present with endocrine abnormalities such as insulin resistance [7] and, although less common than DM1, testicular failure may also be present [5]. In addition to the common clinical features, DM2 and DM1 also share muscle pathological features [8]. Despite the clinical and histological similarities of DM2 and DM1 there are some important differences: DM2 lacks the congenital and juvenile form of DM1; and mental retardation, a prominent feature of the congenital and juvenile form of DM1, is rare in DM2 [3]. Moreover, in DM1 the increased expansion size of the CTG trinucleotide repeats is associated with an earlier age of onset and more severe clinical phenotype [9], whereas in DM2 there is no definite correlation between repeat length and disease severity [6]. DM2 does not show anticipation, i.e., increased length of repeats with worse clinical course in the next generation. DM2 patients seek medical attention mostly because of muscle pain along with stiffness and fatigue, which can develop before symptomatic proximal muscle weakness appears [4], whereas DM1 patients usually present with symptomatic distal weakness [10]. This difference probably explains the usual delay in diagnosing DM2.

Another important difference between the two clinical presentations is the presence of myotonia at the time of diagnosis. Myotonia is defined as slowed relaxation after muscle contraction; it is most prominent in the early stages of DM1, is aggravated by cold and stress, and is most obvious in facial and hand intrinsic muscles [8]. Myotonia is universally present in DM1 patients, whereas in DM2 it is found in about 75% of patients at the time of diagnosis, irrespective of age [4]. In addition, myotonia occurring in DM2 varies in severity, with patients often reporting myotonia-free periods of days to weeks [5]. In our study, only 2 of the 10 patients came to medical attention because of myotonia, while 8 patients had symptomatic proximal muscle weakness without myotonia. Hence, it appears that DM2 may present with proximal muscle weakness without myotonia.

EMG is a useful tool for demonstrating myotonia when this symptom is not detected clinically. Electrical myotonia typically consists of repetitive discharges of muscle fiber action potentials at 20–80 Hz that 'wax and wane' in amplitude and frequency. In general, myotonia is easier to elicit in DM1 than in DM2 [11]. In our study, myotonia was elicited in five patients on the first EMG examination and in all nine studied patients on the second examination. Only in rare instances is clinical or electrical myotonia absent in patients with DM2 [12].

All subjects in our series were of European ancestry: one was from Bulgaria of Sephardic* descent, and nine were Ashkenazi Jews. Until now, all DM2 patients had been from Europe or had European ancestry [2]. In Germany, DM2 is as common as DM1 [13].

We conclude that DM2 often presents with muscle weakness without clinical myotonia. EMG may not show myotonic discharges initially, hence the need for a repeat test. We believe that the disease is underdiagnosed. DM2 should be included in the differential diagnosis of every adult patient with European ancestry presenting with proximal lower limb weakness.

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*Sephardic refers to Jews of North African or Middle Eastern descent, and Ashkenazi to Jews of East European descent

Acute Amiodarone Liver Toxicity Likely Due to Ischemic Hepatitis

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ABSTRACT: **Background:** Hepatotoxicity due to intravenous amiodarone (HIVAD) is a rare side effect with a distinct pattern of enzyme disturbances compared to liver damage from oral amiodarone. Intravenous amiodarone is administered for acute arrhythmias often causing heart failure. The enzyme abnormalities and clinical setting are very similar to that of ischemic hepatitis, a far more common condition.

Objectives: To ascertain if acute HIVAD exists as a separate entity or whether reported cases may be explained by ischemic hepatitis.

Methods: In this case-control retrospective study the files of hospitalized patients with markedly elevated aminotransferases were reviewed for the diagnoses of HIVAD or ischemic hepatitis. Medline was searched for published cases of HIVAD. Pooled data of all patients with HIVAD were compared to a control group with ischemic hepatitis.

Results: There were no significant differences in the clinical characteristics, laboratory results or histological findings between HIVAD and ischemic hepatitis patients.

Conclusions: In our opinion, there is currently insufficient data to support the existence of distinct HIVAD, and ischemic hepatitis is a more probable diagnosis in most reported cases. Withdrawing amiodarone because of assumed hepatic damage could deprive patients of a life-saving therapy.

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KEY WORDS: amiodarone, hepatotoxicity, liver, ischemic hepatitis

Amiodarone is a widely used anti-arrhythmic drug, not uncommonly causing hepatotoxicity when given as chronic oral treatment. The elevation of enzymes is generally chronic, mild and reversible, and less than 1% of the patients require discontinuation of amiodarone due to clinical evidence of hepatitis, which usually develops after more than a year of therapy. Because of the long half-life of the drug, side effects sometimes persist for months even after drug discontinuation. Typical histological findings include steatohepatitis and phospholipidosis [1,2].

A much rarer liver injury, distinct in nature from that associated with chronic oral use, has been described following intravenous amiodarone administration. This disorder is acute, manifesting within 1–3 days following IVAD with markedly elevated aminotransferase levels, histological findings of centrilobular necrosis, and a potentially fatal outcome [3-12]. In most reported cases, the abnormalities resolved within days after the medication was discontinued. A search of the medical literature revealed 22 cases reported over 20 years.

IVAD is administered in patients with preexisting cardiac disease to treat arrhythmias, which reduce cardiac output and may cause hypotension and organ hypoperfusion. These clinical findings are also typical of ischemic hepatitis, a condition resulting from severe hypoxia to hepatocytes, characterized by acute marked elevations of aminotransferase and lactate dehydrogenase levels, which typically normalize within days following circulatory improvement, and by histological findings of centrilobular necrosis [13].

The diagnosis of ischemic hepatitis is often missed due to the complexity of the patients, as well as to confounding causes of liver damage, such as sepsis and the administration of hepatotoxic drugs. The reported incidence is 0.16–1.5% of hospitalized patients. However, ischemic hepatitis is considered the most common cause of markedly elevated aminotransferase levels in hospitalized patients and is responsible for up to 50% of the cases [14].

Physicians in the acute care setting may be reluctant to administer IVAD, with its potential hepatotoxicity, to patients already prone to biochemical liver disturbances resulting from impaired hepatic blood flow secondary to cardiac compromise. Conversely, IVAD is expected to improve hemodynamic status and liver blood supply, thus being actually therapeutic in these situations.

Due to the similar clinical setting and laboratory findings, we suspected that these two conditions – ischemic hepatitis and hepatotoxicity due to intravenous amiodarone – might easily be interchanged. Because of the extreme rarity of acute HIVAD and the distinct pattern of liver abnormalities in HIVAD compared with oral amiodarone, as well as the much higher prevalence of ischemic hepatitis coupled with unawareness of this diagnosis, we questioned the existence of a distinct HIVAD entity.

*** The first two authors contributed equally to this study**

IVAD = intravenous amiodarone
HIVAD = hepatotoxicity due to intravenous amiodarone

To address this question, we examined the possibility of ischemic hepatitis as an alternative diagnosis for patients diagnosed with acute HIVAD. We show almost complete similarity between HIVAD cases and ischemic hepatitis, thus raising doubt regarding HIVAD as a separate entity.

PATIENTS AND METHODS

We conducted a search of the laboratory database of the Tel Aviv Sourasky Medical Center for all patients admitted between January 2005 and December 2006 with markedly elevated aminotransferases and LDH, namely, aspartate and alanine aminotransferases > 500 U/L, LDH > 1000 U/L. All retrieved files were reviewed for diagnosis of HIVAD as well as for the possible diagnosis of ischemic hepatitis according to the criteria of Gibson and Dudley (1984): marked elevation (above 20 times normal) and rapid normalization of hepatocellular enzyme levels, with no other apparent cause for abnormal liver tests, in the appropriate clinical setting, namely, an acute illness with a reduction of blood pressure or of cardiac output. Histological confirmation is not required by these criteria [13].

To better study the characteristics of HIVAD patients, in light of the rarity of this condition and potential scarcity of HIVAD patients in our search, we sought to expand this group to include published cases with HIVAD. We conducted a Medline search using the key words: amiodarone AND liver, hepatotoxicity, hepatitis, hepatic necrosis, hepatic damage. All cases of acute IVAD-induced liver injury in the English literature during the years 1986–2009 were reviewed and included in the study, including all cases cited in the reference list of the published reports.

The available clinical and laboratory data of all patients with HIVAD (pooled from our records and from medical literature) were compared with a group of control patients matched for age and gender, selected from the subgroup that fulfilled the criteria for ischemic hepatitis in the above database. Statistical comparison used Fisher's exact test for the clinical findings and the paired *t*-test for laboratory findings.

Hypotension was defined as less than 90 mmHg or 60 mmHg systolic or diastolic pressures, respectively, or as mean arterial pressure under 70. When measurements of cardiac index or wedge pressure were not available we used clinical features typical of low cardiac output, including arrhythmias accompanied by pulmonary congestion or edema (demonstrated clinically or radiographically), severe oliguria, cool extremities, syncope, circulatory collapse, need for direct current shock or mechanical resuscitation, gross peripheral edema, jugular venous distension of > 5 cm from sternal angle, positive hepatojugular reflex, or congestive hepatomegaly. Renal deterioration was defined as a rise of more than 0.5 mg/dl of plasma creatinine levels. Time to normalization of enzymes was defined as the earlier event between normal levels and an improvement of 90% (1 log) from peak levels.

The standard RUCAM scoring system for assessing drug causality of adverse reactions was applied to the HIVAD group [15,16]. This score was designed specifically for drug-induced liver injury. Points are assigned according to time to onset from the beginning/cessation of the drug, course of biochemical abnormalities, presence of risk factors for drug-induced liver injury, concomitant confounding drugs, adequate ruling out of various non-drug causes, previous information on the hepatotoxicity of the drug, and response to rechallenge, if performed.

LDH = lactate dehydrogenase

RESULTS

A total of 220 patients with markedly elevated liver enzymes were admitted to our medical center between January 2005 and December 2006. A probable diagnosis of ischemic hepatitis could be made in 116 cases. Other common diagnoses included viral hepatitis, biliary disease and liver infiltration. HIVAD was diagnosed by the physicians in three cases, and Medline revealed 22 additional cases of HIVAD published in the English literature. Twenty-five patients from the ischemic hepatitis group matched the HIVAD patients for age and gender. Baseline characteristics and findings of both groups are summarized in Tables 1 and 2, respectively.

Background congestive heart failure was common in both groups. Also common to both were the clinical findings of hypotension, low cardiac output and renal deterioration. Importantly, in all cases, the episode of hypotension and/or low cardiac output occurred before IVAD administration and therefore was not a result of IVAD therapy.

Baseline aminotransferase levels were normal in all cases but one, with an AST of 200 and ALT of 100. IVAD was administered for 1–3 days in all cases. Both groups exhibited dramatic increases in aminotransferase levels and rapid

AST = aspartate aminotransferase
ALT = alanine aminotransferase

Table 1. Baseline characteristics of patients with HIVAD compared to patients with IH

	HIVAD (n=25)	IH (n=25)	*P* value
Patient age (mean yrs)	64.36	64.0	NA
Gender, male, n (%)	14 (56)	14 (56)	NA
Amiodarone dose (total mg)	1518 ± 816		NA
Background			
Congestive heart failure, n (%)	20 (80)	13 (52)	0.04
Renal failure (creatinine › 1.5), n (%)	7 (28)	8 (32)	0.38
Diabetes, n (%)	9 (36)	8 (32)	0.39

HIVAD = hepatotoxicity due to intravenous amiodarone, IH = ischemic hepatitis

Table 2. Clinical and laboratory findings of patients with HIVAD compared to patients with IH

	HIVAD (n=25)	IH (n=25)	*P* value
Clinical findings (no. of patients)			
Documented hypotension, n (%)	14 (56)	18 (72)	0.26
Low cardiac output, n (%)	18 (72)	19 (76)	0.49
Renal deterioration, n (%)	21 (84)	22 (88)	0.71
Laboratory characteristics (mean peak value ± SD)			
AST	2531 ± 2006	2507 ± 1922	0.29
ALT	2035 ± 1698	1762 ± 1050	0.21
LDH	5271 ± 3221*	4260 ± 2430	0.47
Days to normalization (mean ± SD)			
AST	6.2 ± 3.2	5.2 ± 2.5	0.10
ALT	11.6 ± 2.2	10.3 ± 2.7	0.46
LDH	3.8 ± 1.7	4.7 ± 1.2	0.67

*Data lacking in most published cases

HIVAD = hepatotoxicity due to intravenous amiodarone, IH = ischemic hepatitis, AST = aspartate aminotransferase, ALT = alanine aminotransferase, LDH = lactate dehydrogenase

normalization, with no significant differences between the groups in the enzyme values or normalization time. The time to peak aminotransferase levels from IVAD administration, but also from the acute event of hypotension or low cardiac output state to aminotransferase elevation, was 1–3 days in all cases. Most cases reported clinical and enzymatic improvement 1–2 days after IVAD withdrawal and nearly complete resolution in up to 7 days. In two cases enzyme levels normalized despite continuation of IVAD [6].

A positive rechallenge with IVAD was reported in two cases [7,8]. However, in two other cases, rechallenges with IVAD performed following clinical improvement were negative and resulted in normal liver enzymes [9,17].

Concomitant drugs that could influence IVAD toxicity or cause independent hepatotoxicity in the HIVAD group included acetaminophen, phenytoin, statins, metoprolol, furosemide, propofol, captopril, enalapril and aspirin. Most drugs were taken chronically before presentation.

Based on the above data, we next applied the RUCAM algorithm to the HIVAD group to assess the probability of a causal relationship between IVAD administration and the ensuing biochemical hepatocellular disturbance [15,16], assuming ischemic hepatitis could be an alternative cause for liver toxicity. Table 3 lists the diagnostic criteria of the algorithm met by the HIVAD cases.

Assigning the appropriate points designated by the scoring system to each case, 72% of HIVAD cases would obtain a score of 3 points (maximum 14) ("possible reaction"), 12% would score 2 points ("unlikely"), 8% would score 0 points ("ruled out"), and only 8% would score 6 points ("probable"). Of note, this algorithm relies heavily on a suggestive time-course of improvement when discontinuing the drug; however, this is the exact identical course in ischemic hepatitis.

Table 3. Assessment of HIVAD patients to assess drug causality by RUCAM score

Criteria	Results	No.	Assessment score (points)
Time to onset from beginning of drug	Compatible (< 5 days)	25	1
Course after cessation of drug	Highly suggestive (decrease of peak ALT > 50% within 8 days)	23	3
	Inconclusive (drug continued)	2	0
Risk factor for drug reaction	Age ≥ 55 Age ≤ 55	22 3	1 0
Concomitant drug(s)	None/no information/drug with incompatible time to onset	22	0
	Drug with compatible time to onset	3	-1
Non-drug related causes	Probable	25	-3
Previous information on hepatotoxicity of drug	Reaction published but unlabeled	25	1
Response to rechallenge	Positive Negative Not done	2 2 21	3 -2 0

HIVAD = hepatotoxicity due to intravenous amiodarone, ALT = alanine aminotransferase

A more recent algorithm weighs the same criteria with reference to two groups of senior experts' opinion and provides discrete probabilities of drug causation [18]. When applied to the HIVAD cases, the probabilities are 27%, 38%, and 50% for 24%, 68%, and 8% of the patients, respectively. Thus, 92% of the patients have a probability that is less than neutral.

Liver biopsy was performed in four HIVAD cases. Three demonstrated centrilobular necrosis [11,12], the typical histology for ischemic hepatitis, while one showed atrophy of hepatocytes with a granulocytic and eosinophilic infiltrate [8].

DISCUSSION

HIVAD has been accepted as a distinct cause for acute amiodarone-induced liver damage in major gastroenterology, hepatology and clinical pharmacology textbooks [19-21]. Therefore, the clinician treating patients suffering from acute heart failure and arrhythmia, who often have some degree of liver enzyme elevation, may be faced with the dilemma of whether to administer a drug that could potentially exacerbate liver biochemical abnormalities.

The criteria that could potentially establish a causal relationship between amiodarone and hepatotoxicity are exclusion of other drug- and non-drug-related causes, suggestive time to onset, improvement upon drug withdrawal, positive rechallenge, presence of risk factors for drug hepatotoxicity,

and the reaction being documented and labeled. Different algorithms are provided to determine the probability for a causal relationship between a drug and an adverse effect according to these criteria [15,16,18].

We suggest that most of the patients' findings could be explained by ischemic hepatitis, since the HIVAD patients fulfilled all the criteria required for diagnosis of ischemic hepatitis, at a similar or even increased incidence compared to the ischemic hepatitis group: an acute, marked elevation in aminotransferases, with rapid normalization upon circulatory improvement [22], decompensated heart failure, hypotension, low cardiac output and renal deterioration. Although liver histology is not required for the diagnosis of ischemic hepatitis, centrilobular necrosis – the characteristic finding in ischemic hepatitis – was present in the HIVAD group where histology was available.

Congestive heart failure was a common background diagnosis in both groups, more so in the IVAD group. This difference is understandable since IVAD is administered primarily to patients with known heart disease and arrhythmias, whereas ischemic hepatitis occurs in patients with acute liver hypoxia from various etiologies [23-25].

Some authors describing HIVAD rejected the possibility of ischemic hepatitis due to lack of documented hypotension. However, a very brief, easily overlooked hypotensive episode – 15 minutes – has been shown to be sufficient for causing ischemic hepatitis [23]. Numerous cases of ischemic hepatitis in the absence of bona fide hypotension have been reported, involving other causes of transient liver hypoperfusion, such as hypoxia, anemia or arrhythmias causing decreased cardiac output and relative hypotension, superimposed on right-sided heart failure with hepatic congestion [13,24,25].

The time to onset in most HIVAD cases (1–3 days), although compatible with a drug cause, is not suggestive of it [15]. However, it is highly indicative of ischemic hepatitis, which typically occurs acutely [13]. Age, considered a predominant risk factor for drug hepatotoxicity in these reported cases, is also a risk factor for ischemic hepatitis [23].

Most cases report rapid enzymatic improvement upon IVAD withdrawal. This is despite a drug half-life of 15–100 days and in contrast to hepatotoxicity from oral amiodarone which may consequently persist for months. We suggest that concurrent improvement in cardiovascular status and liver perfusion causes resolution of ischemic hepatitis and is responsible for this rapid improvement. This may also explain why in some cases enzyme levels normalized despite continued IVAD [6], while in others rechallenge with IVAD after circulatory improvement was negative [9]. Although rechallenge was positive in two cases [8,9], retreatment in both was due to recurrent arrhythmia, which may have caused an additional bout of ischemic hepatitis.

According to the algorithms we applied for causality assessment of drug-induced injuries, the vast majority of HIVAD cases would obtain a score of "possible drug reaction" at the most, or if quantified less than 50%.

A potential mechanism of HIVAD could be liver ischemia due to hypotension or an unknown mechanism. This would make the distinction between HIVAD and ischemic hepatitis very difficult, and perhaps discontinuation of IVAD appropriate. We cannot completely rule this out; however, in the clinical setup of arrhythmia causing decreased cardiac output, amiodarone is much more likely to improve liver perfusion, rather than worsen it, by correcting the rhythm problem and the cardiac output. In addition, when documented, hypotension always preceded IVAD administration rather than being a result of the drug administration.

Our study has several shortcomings. First, it was retrospective; therefore, not all the required data were available for all patients. Second, due to the small number of cases in our search, we had to broaden the group by including published cases, which provided less detailed data. Lastly, objective measurements of low cardiac output were lacking in many cases, requiring us to rely on clinical features.

In conclusion, medical background, clinical characteristics, and laboratory and histological findings in almost all cases with supposed HIVAD were strikingly similar to those with ischemic hepatitis. Presumptive HIVAD is exceedingly rare, while ischemic hepatitis is the most common cause of markedly elevated aminotransferases, particularly in patients with chronic cardiovascular illnesses. Given that HIVAD by an ischemic or other mechanism cannot completely be ruled out, it is our opinion that a patient presenting with an arrhythmia and acute low cardiac output, who develops a rise in aminotransferases after receiving IVAD, is much more likely to have ischemic hepatitis than HIVAD. Correcting the arrhythmia with IVAD treatment may increase liver blood flow and ameliorate ischemic hepatitis. Thus, reluctance on the part of the clinician to use amiodarone, or withdrawal of the drug when biochemical liver abnormalities appear, could deprive patients of an important and sometimes life-saving therapy. The possible risk and benefit of continuing or discontinuing amiodarone should be weighed in every case.

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Capsule

Clearance of p16Ink4a-positive senescent cells delays aging-associated disorders

Advanced age is the main risk factor for most chronic diseases and functional deficits in humans, but the fundamental mechanisms that drive aging remain largely unknown, impeding the development of interventions that might delay or prevent age-related disorders and maximize healthy lifespan. Cellular senescence, which halts the proliferation of damaged or dysfunctional cells, is an important mechanism to constrain the malignant progression of tumor cells. Senescent cells accumulate in various tissues and organs with aging and have been hypothesized to disrupt tissue structure and function because of the components they secrete. However, whether senescent cells are causally implicated in age-related dysfunction and whether their removal is beneficial has remained unknown. To address these fundamental questions, Baker and co-authors made use of a biomarker for senescence, p16Ink4a, to design a novel transgene, INK-ATTAC, for inducible elimination of p16Ink4a-positive senescent cells upon administration of a drug. They show that in the BubR1 progeroid mouse background, INK-ATTAC removes p16Ink4a-positive senescent cells upon drug treatment. In tissues – such as adipose tissue, skeletal muscle and eye – in which p16Ink4a contributes to the acquisition of age-related pathologies, life-long removal of p16Ink4a-expressing cells delayed onset of these phenotypes. Furthermore, late-life clearance attenuated progression of already established age-related disorders. These data indicate that cellular senescence is causally implicated in generating age-related phenotypes and that removal of senescent cells can prevent or delay tissue dysfunction and extend health span.

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Eitan Israeli

"To sin by silence when they should protest makes cowards of men"

Abraham Lincoln (1809-1865), 16th President of the United States

"Facts do not cease to exist because they are ignored"

Aldous Huxley (1894-1963), English writer best known for his novel *Brave New World*; he also published essays, short stories, poetry, travel writing and film scripts. Huxley was a humanist and pacifist, and was latterly interested in spiritual subjects such as parapsychology and philosophical mysticism

High Yield of Oocytes without an Increase in Circulating Estradiol Levels in Breast Cancer Patients Treated with Follicle-Stimulating Hormone and Aromatase Inhibitor in Standard Gonadotropin-Releasing Hormone Analogue Protocols

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ABSTRACT: **Background:** Adjuvant/neoadjuvant chemotherapy in breast cancer patients may be associated with amenorrhea and a marked reduction in ovarian reserve.

Objectives: To assess the use of letrozole with follicle-stimulating hormone (FSH) in gonadotropin-releasing hormone (GnRH) analogue protocols, based on reported attempts to avoid the estradiol (E2) increase during controlled ovarian hyperstimulation for embryo cryopreservation in breast cancer patients using a combination of low dose FSH and aromatase inhibitor (letrozole) in a GnRH-antagonist protocol.

Methods: Twenty-four breast cancer patients were treated with recombinant FSH (150–450 U/day) and letrozole (5 mg/day) in a long GnRH-agonist (n=7) or GnRH-antagonist (n=17) protocol. After oocyte retrieval, insemination and/or intracytoplasmic sperm injection was performed. The embryos were frozen.

Results: The average interval from surgery to oocyte retrieval was 40 days. Average duration of treatment was 9.6 days and mean peak E2 level 1342 ± 1091 pmol/L, yielding 16.0 ± 16.3 oocytes (range 0–82). Mean fertilization rate was 69.5 ± 20.4% and mean number of embryos cryopreserved 10.3 ± 9.3. More oocytes were retrieved with the long GnRH protocol, but the difference was not statistically significant (24.8 ± 24.6 vs. 12.0 ± 8.8 pmol/L, $P = 0.07$).

Conclusions: As previously reported, ovarian stimulation with letrozole and FSH, in both the long GnRH-agonist and GnRH-antagonist protocols, is apparently effective in breast cancer patients and spares them exposure to high E2 levels.

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KEY WORDS: breast cancer, in vitro fertilization (IVF), letrozole, fertility preservation

Breast cancer is the most common cancer in women in developed countries. Approximately 2% of cases occur in women aged 20–34 years and 11% in women aged 35–44 years [1]. With the significant improvement in the survival of breast cancer patients in recent years, the potential late effects of treatment and the impact on quality of life have become increasingly important. Adjuvant/neoadjuvant chemotherapy may be associated with amenorrhea and a marked reduction in ovarian reserve, depending on the patient's age and the class and dose of drugs used [2-4]. Moreover, in patients with estrogen-positive disease, the need to delay pregnancy for several years during hormone treatment (tamoxifen with or without gonadotropin-releasing hormone agonists) places an additional burden on the already diminished ovarian reserve.

To avoid the potential risks of rising estradiol levels during controlled ovarian hyperstimulation in women with breast cancer, Oktay et al. [5-7] described an ovarian stimulation protocol where the aromatase inhibitor letrozole was administered before in vitro fertilization for embryo or oocyte cryopreservation. They found that E2 remained at levels similar to those in unstimulated cycles, and the oocyte and embryo yields were comparable to those of standard ovarian stimulation protocols. Since these patients usually undergo only a single IVF attempt before commencing chemotherapy, it is crucial that as many cryopreserved embryos as possible be obtained in this cycle for future use.

In their pioneer study, Oktay and co-authors [6] used a low dose (150 U/day) of recombinant follicle-stimulating hormone in a GnRH-antagonist protocol. The aim of the present study was to evaluate the combination of letrozole with higher doses of FSH in long GnRH-agonist and GnRH-antagonist protocols.

E2 = estradiol
IVF = in vitro fertilization
GnRH = gonadotropin-releasing hormone

PATIENTS AND METHODS

The study population consisted of patients with breast cancer who between 2005 and 2009 were referred for consultation for fertility preservation before adjuvant chemotherapy. All data were collected prospectively by one physician (A.B-H.). The present retrospective analysis was approved by the local institutional review board. Only women with stage III cancer or lower were treated.

Ovarian stimulation was performed with recombinant FSH (Gonal F, Serono, Geneva, Switzerland) at a starting dose of 150–375 IU/day in a long mid-luteal GnRH-agonist or GnRH-antagonist protocol. The choice of protocol was made on an individual basis by the treating physician (A.B-H.) according to the expected time of menstruation. The long GnRH protocol consisted of daily injections of Decapeptyl® (Ferring, GmbH, Kiel, Germany) 0.1 mg or a depot injection of Decapeptyl 3.75 mg at the mid-luteal phase. Down-regulation was confirmed after menstruation and was followed by gonadotropin stimulation. The GnRH-antagonist protocol consisted of daily gonadotropin stimulation from day 3 or 4 of menstruation followed by daily injections of Cetrotide® 0.25 mg (Serono) when the leading follicle reached 14 mm and continued until the day of human chorionic gonadotropin injection. Letrozole (Femara®, Novartis Pharma Sein AG, Basel, Switzerland) was started at 5 mg/day on the second day of the menstrual cycle and continued until the day of hCG trigger. The starting gonadotropin dose was determined on the basis of the patient's age and body mass index according to our standard departmental protocols. A baseline pelvic ultrasound assessment was performed on cycle day 2, and ultrasound and E2 monitoring were then performed every 2 to 4 days after the initiation of gonadotropins. Intramuscular hCG was administered when at least two follicles reached at least 18–20 mm in diameter. Transvaginal oocyte retrieval was performed approximately 36 hours after hCG administration. Oocytes were fertilized by intracytoplasmic sperm injection or standard IVF insemination, depending on the semen parameters. Embryos were cryopreserved by slow freezing at the two-pronucleus stage. Letrozole was reinitiated on the day of oocyte retrieval to prevent a rebound increase in E2 levels and continued for 2–4 days thereafter.

Data were managed and analyzed with the SPSS statistical package, version 15 for Windows. Mann-Whitney and chi-square tests were used, as appropriate. A *P* value less than 0.05 was considered significant.

Table 1. IVF cycle characteristics in breast cancer patients in the long GnRH agonist and GnRH-antagonist protocols

	Long GnRH-agonist protocol (n=7)	GnRH-antagonist protocol (n=17)	*P* value
Age (yrs) (range)	31.8 ± 3.3 (28–39)	32.2 ± 4.6 (24–41)	0.84
Total FSH dose (IU)	2971 ± 1125	2541 ± 1019	0.34
Duration of stimulation (days)	10.5 ± 2.6	9.2 ± 1.8	0.20
FSH dose/day (IU) (range)	275 ± 45 (225–337)	269 ± 80 (150–450)	0.84
Peak estradiol (pmol/L) (range)	1466 ± 1047 (80–3187)	1248 ± 1138 (244–5000)	0.70
No. of retrieved oocytes (range)	24.8 ± 24.6 (0–82)	12.0 ± 8.8 (3–31)	0.07
No. of retrieved oocytes* (range)	± 9.6 (0–30)	12.0 ± 8.8 (3–31)	0.28
Fertilization rate (%)			
IVF	60.6 ± 27.9	58.6 ± 33.5	0.88
ICSI	73.0 ± 15.2	74.7 ± 27.1	0.89
Total	74.7 ± 27.1	73.0 ± 22.0	0.18
No. of frozen embryos	14.2 ± 13.3 (0–45)	8.2 ± 6.4 (2–27)	0.66
No. of frozen embryos*	9.8 ± 5.1 (0–16)	8.2 ± 6.4 (2–27)	0.54

*Excluding one patient who hyper-responded to gonadotropins with 82 retrieved oocytes
ICSI = intracytoplasmic sperm injection

RESULTS

Between 2005 and 2009, 24 patients with breast cancer (mean age 32.1 ± 4.1 years, range 24–41) underwent ovarian stimulation with gonadotropins and letrozole in a long GnRH-agonist protocol (n=7) or a GnRH-antagonist protocol (n=17) [Table 1]. The mean interval from the definitive surgery to oocyte retrieval was 40 days (range 22–49). Patients were treated for an average of 9.6 days, with peak E2 levels of 1342 ± 1091 pmol/L (range 80–5000), yielding a mean of 16.0 ± 16.3 oocytes/cycle (range 0–82). In one woman, no oocytes were retrieved. The overall rate of fertilization was 69.5 ± 20.4%; a mean of 10.3 ± 9.3 embryos (range 0–45) was cryopreserved. One woman on the long GnRH-agonist protocol hyper-responded to gonadotropins (225 IU/day of FSH injections) with 82 retrieved oocytes (peak E2 3187 pmol/L). Ovarian hyperstimulation syndrome was ruled out on clinical, sonographpic and laboratory follow-up. The number of retrieved oocytes was higher in women in the long GnRH protocol than the GnRH-antagonist protocol, but the difference was not statistically significant [Table 1]. During follow-up of 20–52 months, cancer recurrence rate was found in 2 of 24 patients.

DISCUSSION

Based on previous reports, the present study showed that ovarian stimulation with recombinant FSH and letrozole seems to be effective in patients with breast cancer in both the long GnRH agonist and GnRH-antagonist protocols, while preventing the surge in E2 levels. The major limitations in the

HCG = human chorionic gonadotropin

current study were the small sample size and the lack of a control group. However, since a real control group (without aromatase inhibitor) cannot be treated for obvious reasons and a larger number of young breast cancer patients who wish for fertility preservation is difficult to achieve, our study still adds important data to those previously reported by others.

Oktay et al. [6] were the first to describe the use of letrozole in the GnRH-antagonist protocol in a study of 29 patients with breast cancer. Their analysis included 33 ovarian stimulation cycles with tamoxifen 60 mg/day alone, tamoxifen 60 mg/day in combination with low dose FSH, or letrozole 5 mg in combination with low dose FSH (150 U/day). They found that the patients given letrozole and FSH had more follicles, more mature oocytes (8.5 ± 1.6), and more embryos (5.3 ± 0.8) than the other groups. As expected, peak E2 levels were lower with letrozole (1370 ± 205 pmol/L). In our study, patients in the GnRH-antagonist group were treated with higher doses of FSH (150–450 U/day, mean 270 U/day). This resulted in a higher number of retrieved oocytes (12.0 ± 8.8) and frozen embryos (8.2 ± 6.4) than the lower-dose schedule [6] but similarly low levels of peak E2 (1248 ± 1138 pmol/L). The mean total FSH dose was 2697 ± 1050 U, which is higher than reported by Azim et al. [8] (1469 ± 741 U). The long GnRH-agonist protocol was associated with a slightly higher yield of retrieved oocytes and frozen embryos; but the difference from the GnRH-antagonist protocol was not statistically significant [Table 1].

One major concern is the long-term safety of IVF in patients with breast cancer. In their first study, Oktay and team [6] followed their patients for a mean duration of 554 ± 31 days (range 153–1441 days). The cancer recurrence rate was similar in the IVF and control groups (3/29 vs. 3/31 patients, respectively; hazards ratio 1.5, 95% confidence interval 0.29–7.4). The risk was not affected by cancer stage. In a recent follow-up report [9], cancer recurrence was compared among 79 women who elected to undergo ovarian stimulation with letrozole and gonadotropins for embryo or oocyte cryopreservation and 136 patients in whom no fertility-preserving procedure was performed (controls). The median follow-up after chemotherapy was 23.4 months in the study group and 33.05 months in the control group. The hazards ratio for recurrence after IVF was 0.56 (95% CI 0.17–1.9), and the survival rate was similar in the two groups. Given the similar peak E2 levels in our patients to those reported in these studies, we expect the long-term results to be the same.

Since most women undergo only one IVF cycle prior to chemotherapy, a higher yield of oocytes and embryos is desirable for fertility preservation. However, lower FSH doses are usually used in first cycles because of the risk of ovarian hyper-response and OHSS. On the basis of the present findings and the reports in the literature, we recommend the use of letrozole and FSH in the GnRH-antagonist protocol, so that GnRH-agonist can be used instead of hCG for final maturation of oocytes before oocyte retrieval [10]. In this manner, higher doses of FSH can be administered while avoiding the risk of OHSS [11], achieving a higher number and percentage of mature oocytes and a higher number of cryopreserved embryos or oocytes compared with hCG [12]. Importantly, pituitary suppression with a GnRH antagonist may result in a plateau or decrease in estradiol levels [13], another possible advantage in breast cancer patients. Since estradiol levels cannot be used for monitoring the magnitude of ovarian stimulation, frequent ultrasound evaluation of follicle count and size should be performed to prevent exaggerated ovarian hyper-response and OHSS, particularly in younger lean patients with high pretreatment antral follicle count.

It should be emphasized that the current use of letrozole in Israel for this indication is "Off Label," since in 2005 the manufacturer, Novartis Pharmaceutical, issued a statement to physicians advising that the use of letrozole in premenopausal women – and specifically its use for ovulation induction – is contraindicated. This warning was released following the 2005 annual meeting of the American Society for Reproductive Medicine, where an abstract presentation examined a relatively small number of letrozole pregnancies compared with a large control group of spontaneous conceptions [14]. The presenter suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac malformation in newborns. However, more recently, Tulandi et al. [15] evaluated the incidence and type of congenital malformation among 911 newborns of mothers who had conceived with letrozole compared with a control group of infertile women who had conceived with clomiphene citrate. Their study demonstrated no difference in the overall rates of malformations or chromosomal abnormalities among the newborns of mothers who had conceived after letrozole or after clomiphene citrate treatments. Congenital cardiac anomalies in their study were statistically significantly less frequent in the letrozole group than in the clomiphene citrate group. Based on their data, the concern that letrozole use for ovulation induction could be teratogenic is unfounded [16].

In summary, despite the limitation of the low number of patients in the current study, as previously reported by others FSH can be used in IVF cycles for fertility preservation in patients with breast cancer when the potent aromatase inhibitor letrozole is added. This combination yields a high number of oocytes with low peak estradiol levels in both the long GnRH-agonist and GnRH-antagonist protocol, while sparing patients' exposure to high E2 levels.

CI = confidence interval
OHSS = ovarian hyperstimulation syndrome

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Capsule

A *Burkholderia pseudomallei* toxin inhibits helicase activity of translation factor eIF4A

The gram-negative bacterium *Burkholderia pseudomallei*, the causative agent of melioidosis, is endemic in Southeast Asia and northern Australia and is often associated with stagnant water and rice paddy fields. Clinical manifestations of melioidosis include subclinical infections, acute septicemia, and subacute and chronic disease. There is no licensed vaccine against *B. pseudomallei*, which can infect almost any tissues of its hosts and is resistant to a number of antibiotics. Cruz-Migoni et al. report the identification and molecular characterization of a *B. pseudomallei* protein that can act as a potent toxin in mice and human cells and can inhibit protein translation. Expression levels of bpsl1549 correlate with conditions expected to promote or suppress pathogenicity. BPSL1549 promotes deamidation of glutamine-339 of the translation initiation factor eIF4A, abolishing its helicase activity and inhibiting translation. The authors propose to name BPSL1549 Burkholderia lethal factor 1.

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Eitan Israeli

Capsule

Clinical features, pathogenesis and treatment of juvenile and adult dermatomyositis

Juvenile and adult dermatomyositis have multiple commonalities, yet display differing prevalence of features, outcomes and comorbidities. In general, compared with the disease in adults, children with dermatomyositis have more vasculopathy and a greater likelihood of calcinosis, periungual and gingival telangiectasias, and ulceration, but have a better long-term prognosis with improved survival. Adults with dermatomyositis are more likely to have myositis-specific antibodies, develop interstitial lung disease, have amyopathic disease, as well as a marked association with malignancy and other comorbidities. Both diseases have similar features on muscle biopsy and interferon gene signature, although subtle differences can exist in pathogenesis and pathology, such as more capillary loss and a greater degree of C5b-9 complement deposition in affected muscle of juvenile patients. Initiatives are underway to improve classification, markers of disease activity and ability to predict outcome of juvenile and adult dermatomyositis.

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Eitan Israeli

Septic Arthritis of the Knee Following Intraarticular Injections in Elderly Patients: Report of Six Patients

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ABSTRACT: **Background:** Intraarticular injections for the local treatment of osteoarthritis are widely used in the office or hospital setting. Septic arthritis is a potential catastrophic complication of intraarticular injection, as bacterial arthritis of any cause is associated with up to 15% mortality and residual impairment of joint function in up to 50% of survivors. There is lack of evidence regarding the precautions that should be taken to avoid such a complication, as well as how often it is encountered.

Objectives: To report our experience with the clinical presentation, diagnosis and treatment of knee septic arthritis following intraarticular injections.

Methods: We followed six patients who were admitted to the hospital and underwent surgery for the treatment of pyogenic arthritis following injection to the knee joint in outpatient clinics.

Results: All but one patient were over 70 years old with comorbidities. Three patients were injected with steroid preparations and three with hyaluronic acid several days before admission. In all six patients the infection was treated surgically and three of them had undergone more than one operation during their hospitalization. Four of the six patients were treated by means of an open arthrotomy and synovectomy, and the other two were treated successfully with arthroscopic lavage and synovectomy. One patient underwent an above-knee amputation due to septic shock and died after several days.

Conclusions: Despite the rarity of this complication, surgeons must be aware of the possibility of pyogenic arthritis when administering injections, especially in elderly patients with serious underlying medical conditions.

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KEY WORDS: septic arthritis, knee, injection, corticosteroids, hyaluronic acid, elderly

Intraarticular injections of corticosteroids and hyaluronic acid to the knee are widely practiced as a conservative treatment modality for osteoarthritis [1-4]. However, there are certain side effects related to intraarticular injections, most commonly with corticosteroids. The most common side effects of corticosteroid injections are postinjection flare, facial flushing, and skin or fat atrophy of the injection site [5-7].

The overall incidence of side effects of hyaluronic acid injections, also referred to as viscosupplementation, has been reported to be around 1% to 3% per injection. The most common adverse event is an injection-site inflammatory reaction of the treated knee (i.e., pain, warmth, swelling) that lasts from 1 to 2 days [8,9]. Of the less common side effects, pyogenic arthritis is one of the greatest concerns, with reported incidences ranging from 1 in 3000 to 1 in 50,000 following corticosteroid injection [6]. Improved antiseptic techniques and availability of corticosteroid preparations in prefilled syringes may have even lowered the incidence. In a survey of 191 orthopedic surgeons, rheumatologists and general practitioners, only 12.6% had ever encountered septic arthritis after corticosteroid injection of the knee, and only 3% had encountered it more than once [4].

Septic arthritis following hyaluronic acid injections seems rare, but has been described [10-12]. A deep infection following an intraarticular injection may develop due to direct inoculation of bacteria by the injection, to hematogenous seeding of the percutaneous injection tract, or to activation of quiescent infection by an injected steroid [4]. The most common organism encountered is *Staphylococcus aureus*, with occasional involvement of other organisms, including coagulase-negative staphylococci and anaerobes [13]

Prompt recognition and treatment of the infected joint is critical for a successful outcome. The cornerstones of treatment include early diagnosis, pathogen identification by synovial fluid cultures, surgical debridement and tailored antibiotic therapy. Irrigation and debridement of the knee joint may be accomplished by either a formal arthrotomy or the arthroscopic technique. In addition to proper debridement of the infected joint, it is important to obtain cultures during surgery. Beginning immediate treatment with antibiotics even before culture results are obtained is of paramount importance.

PATIENTS AND METHODS

This study is a case series where all the clinical, laboratory and intraoperative findings were collected retrospectively.

Table 1. Patient demographics and comorbidities, the injected preparation and presenting symptoms

Patient	Gender	Age (yrs)	Comorbidities	Preparation injected	Time from injection to symptoms (days)	Presenting symptoms and physical exam
1	F	87	IHD, hyperlipidemia	Corticosteroid	2	No fever, pain, swelling
2	M	86	CAF,COPD HTN, IHD, CHF, CRF	Corticosteroid	1	No fever, chills, pain, swelling
3	F	71	DM, hyperlipidemia	Corticosteroid	3	No fever, pain, swelling, difficulty walking
4	M	70	Hyperlipidemia	Hyaluronic acid	2	Subfebrile fever, pain, swelling
5	F	75	Asthma, IHD hyperlipidemia	Hyaluronic acid	2	No fever, pain, swelling
6	F	64	HTN, pulmonary hypertension, MVR	Hyaluronic acid	5	No fever, pain, swelling

IHD = ischemic heart disease, CAF = chronic atrial fibrillation, COPD = chronic obstructive pulmonary disease, HTN = hypertension, CHF = congestive heart failure, CRF = chronic renal failure, DM = diabetes mellitus, MVR = mitral valve replacement

Table 2. Blood tests and synovial fluid analysis at the time of diagnosis

	Serum laboratory values				Synovial fluid analysis		
Patient	WBC	NEUT%	ESR	C-reactive protein	WBC k/µl	NEUT%	Smear
1	15.77	63.6	>100	11.84	60,000	88	–
2	7.16	82	55	–	50,000	85.7	–
3	14.69	80	15	17	105,000	91	+
4	12.2	72.4	50	16	68,030	91.8	–
5	10.37	90.6	–	17.64	28,250	96.5	+
6	12,450	77	–	33.56	82,250	93	–

WBC = white blood cells, NEUT = neutrophils, ESR = erythrocyte sedimentation rate

Table 3. The organism cultured, antibiotic and surgical treatment

Patient	Causative microorganism	Antibiotic treatment	Surgical treatment (days from admission)
1	*Streptococcus viridans*	IV cloxacillin	Arthrotomy (1)
2	MSSA*	IV cloxacillin	Arthrotomy (1) Arthrotomy (18) Above-knee amputation (34)
3	*Streptococcus oralis*	IV vancomycin + PO ciprofloxacin	Arthrotomy (1) Arthrotomy (3) Arthrotomy (14)
4	–	IV cefazoline	Arthroscopic lavage and synovectomy (1) Arthroscopic lavage and synovectomy (7)
5	Staphylococcus coagulase-positive	IV cloxacillin + PO rifampicin	Arthroscopic lavage and synovectomy (8)
6	Staphylococcus coagulase-negative	IV cefazoline	Arthrotomy (15)

*MSSA = methicillin-sensitive Staphylococcus aureus, IV = intravenous, PO = per os

Institutional Ethics Committee approval was obtained for this study in March 2011. We included all patients diagnosed with septic arthritis of the knee following an intraarticular injection in an outpatient clinic for the treatment of osteoarthritis. Between September 2006 and January 2010 we treated six patients diagnosed with septic arthritis of the knee following intraarticular injection. Demographic patient data are presented in Table 1. All six patients were admitted within a few days of receiving an injection, and all were diagnosed with monoarticular septic arthritis of the injected knee.

RESULTS

The mean age was 75 years (range 64–87). Most of the patients had significant comorbidities, as summarized in Table 1. Three of the patients were treated with a corticosteroid preparation, the other three with hyaluronic acid.

All patients arrived at the emergency room 1 to 5 days after the injection. One patient (# 3) was transferred to our medical center from another hospital where she was diagnosed and underwent her first operation. The main presenting complaints [Table 1] were pain, swelling of the involved limb and difficulty walking. All patients had general malaise at the time of presentation to the emergency room but none of the patients had fever. One patient had a subfebrile fever and another patient had chills.

Physical examination of all six patients revealed swelling of the involved knee, the presence of an effusion, and pain on active and passive movement. Blood test results and synovial fluid analysis on admission are shown on Table 2. Four patients had a mildly elevated white blood count. Most of the patients had elevated sedimentation rate or C-reactive protein. The synovial fluid analysis revealed a white blood cell count of above 50,000 in most of the patients, with a high percentage of neutrophils. Only two had a positive Gram stain on direct microscopy at admission.

The causative bacteria on synovial fluid culture were staphylococci or streptococci. One patient (# 4) had a sterile culture, probably due to treatment with oral antibiotics before admission, prescribed by his primary care physician. Once synovial fluid analysis and cultures were obtained, we began intravenous antibiotic treatment with cefazolin. Pathogen-specific therapy was tailored after culture results were received. The cultured microorganisms and antibiotic treatment administered to each patient are summarized in Table 3.

All patients had undergone surgical treatment. In four patients surgical intervention was performed within the first 24 hours of admission. Patient 6, a 64 year old woman, refused surgery during the first 2 weeks of hospitalization and was therefore initially treated only with intravenous antibiotics. Since her condition did not improve, she finally gave consent and underwent an arthrotomy. Patient 5, a 75 year old woman,

was diagnosed with septic arthritis during a hospitalization in the internal medicine department where she was admitted due to another medical condition. She was immediately transferred to our unit and underwent prompt surgical debridement. One patient (# 3) was transferred to our hospital after being diagnosed and treated surgically in another medical center. This patient eventually needed further surgical debridements, the first of which was performed 3 days after her admission.

The type of surgery, the number of surgical interventions and the time intervals between surgeries for each patient are summarized in Table 3. Three of the six patients had undergone more than one surgery. Four patients were treated with formal arthrotomy and two were treated successfully with arthroscopy. The intraoperative findings in all cases included congested and thickened synovium with a purulent material. We used surgically placed postoperative drains in all six patients. One patient (# 2), an 86 year old man, was admitted to the intensive care unit postoperatively after his second arthrotomy due to progressive sepsis. Later on he developed septic shock. He was taken once again to the operating room for an urgent above-knee amputation. He continued to deteriorate and eventually died in the ICU from septic shock and multiorgan failure a short time after the surgery.

Postsurgically all patients were treated in the same manner: antibiotics were given for 4 to 6 weeks after surgery, the knees were immobilized for 3 days after surgery and were then put in a continuous passive-motion physiotherapy machine, and the drains were left for several days. The mean duration of hospital stay was 22.5 days (range 9–40). Patients were discharged and continued follow-up in our hospital clinic. The infection finally resolved in five patients.

DISCUSSION

There is a long history of injection of steroids and hyaluronic acid derivatives into joints for the purpose of symptomatic relief and anti-inflammatory effect. However, controversy persists as to whether or not this treatment has any long-term effects and there is no evidence that injection of either corticosteroids or hyaluronic acid alters the natural history of the disease. In a metaanalysis comparing intraarticular injections of corticosteroids with placebo, corticosteroids were probably more effective but their effect was short-lived and lasted only a few weeks [1]. The Cochrane group reported 1 week as the only time point at which a statistically significant difference can be found between corticosteroids and placebo [14].

Intraarticular hyaluronic acid injections have been extensively studied in numerous clinical trials. While not all studies have shown benefit, several meta-analyses of various trials suggested that it provides some modest improvement in patients with knee osteoarthritis [8,15-17].

In a recent multicenter, randomized, double-blind study, Jørgensen et al. [3] compared injections of hyaluronic acid to placebo and found no significant benefit for hyaluronic acid in a 1 year follow-up. Wang and researchers [17] reviewed 20 blinded, randomized, controlled trials that compared intraarticular hyaluronic acid with placebo in patients with knee osteoarthritis. They noted that patients older than 65 years of age and those with the most advanced radiographic stage of osteoarthritis (complete loss of the joint space) were less likely to benefit from intraarticular injection of hyaluronic acid.

In 2009, the American Association of Orthopedic Surgeons published clinical management guidelines for the non-arthroplasty treatment of osteoarthritis of the knee based on a systematic review of published studies [18]. The guidelines suggest that corticosteroids be used for short-term pain relief only. The board could not recommend for or against the use of hyaluronic acid.

We have retrospectively studied six patients who presented to our medical center with pyogenic monoarthritis of the knee following an injection. As we have shown, injections with either corticosteroids or hyaluronic acid may have deleterious consequences, with significant morbidity and even mortality. The six patients were all older adults, with a mean age of 75 years. In this particular age group, the treatment of osteoarthritis may be challenging. Elderly patients are usually treated with a wide array of concomitant medications, and the tolerability of current therapeutic regimens for osteoarthritis must be considered. The potential adverse effects of systemic treatments such as non-steroidal anti-inflammatory drugs in older adults may be devastating. A localized therapy such as intraarticular injections may seem safe and therefore attractive. However, the efficacy of this treatment, as mentioned before, is limited to pain relief only, and for a limited time. Moreover, the treatment of osteoarthritis with intraarticular injections in this specific age group has been shown to be less beneficial [17].

Although quite rare, septic arthritis of the knee may develop following injections to the joint. As we have shown, the treatment of such infections may be prolonged, involving recurrent operations, long-term regimen of antibiotics, and remaining disability. One of our patients, an 87 year old man, did not recover and developed septic shock for which he underwent an urgent below-knee amputation. Unfortunately, he did not survive and eventually died in the intensive care unit. Septic arthritis of the knee may therefore be both a limb-threatening and a life-threatening condition.

When treatment with an intraarticular injection is being considered, the general condition of the patient must be thoroughly assessed, including his or her comorbidities, the degree of osteoarthritis, concomitant drugs used by the patient, past injections and their effect, and history of pre-

ICU = intensive care unit

vious infections. One also should clarify if the patient is a candidate for arthroplasty in the future. If the patient is a candidate for total knee replacement, treatment with intraarticular injections of steroids could put him or her at increased risk for developing a deep infection following arthroplasty, as suggested by Papavasiliou et al. [19]. The decision to administer intraarticular injections to the knee should therefore not be made lightly.

Despite the rarity of septic arthritis following injections, patients should be informed of the risk of septic arthritis and the possible consequences. Furthermore, the increased risk for future deep infections after arthroplasty should be discussed with patients who are possible candidates for total knee replacement.

A full aseptic technique includes proper draping, use of chlorexidine, sterile gloves, and separate needles for drawing and injecting. Studies that examined the precautions taken before intraarticular injections revealed a wide variation among physicians [4]. In an article published in the *Journal of Bone and Joint Surgery* in 1969, Bentley and Goodfellow [20] presented what they called disorganization of the knee joint after injections with hydrocortisone. They concluded: "The case against multiple injections is so strong that the practice should, in our opinion, be discarded, which implies that even a single injection requires strong justification." We totally agree with their statement. Intraarticular injection to the knee is not a harmless procedure and can be both limb-threatening and life-threatening. Furthermore, its long-term benefit is still questionable. We believe that other treatment options should be sought before offering this modality to patients. If administered, it should be done with a strict antiseptic technique.

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"It is of interest to note that while some dolphins are reported to have learned English – up to fifty words used in correct context – no human being has been reported to have learned dolphinese"

Carl Sagan (1934-1996), American astronomer and writer

"Before we set our hearts too much on anything, let us examine how happy are those who already possess it"

Francois, duc de La Rochefoucauld (1613-1680), French moralist

Chronic Fatigue Syndrome: Still a Long Way to Go

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KEY WORDS: chronic fatigue syndrome, fever, Epstein-Barr virus, retrovirus

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Chronic fatigue syndrome is an enigmatic clinical entity defined by persistent, relapsing fatigue associated with substantial impairments of 6 months duration and beyond. An additional four (of eight) secondary symptoms, which cluster into the domains of flu-like pain and neurocognitive symptoms, are required for the diagnosis [1].

During the last three decades, a multitude of papers were published implying various etiologies for the disease. Of the many viruses considered to be the culprit, none was convicted. For example, in 1991, the prestigious journal *Proceedings of the National Academy of Sciences* published a study on the role of HTLV-2 in chronic fatigue syndrome [2], but subsequent studies failed to replicate the results. Almost two years ago, a publication in *Science* claimed that most cases of chronic fatigue syndrome were associated with a newly described gamma retrovirus, xenotropic murine leukemia virus-related virus [3]. This virus was previously detected in human prostate tumor tissue. Since retroviruses are known to affect both neurological and immunological function, Lombardi and colleagues [3] suggested that XMRV may be involved in the pathogenesis of chronic fatigue syndrome. They detected the virus in 67% of patients with the syndrome compared to only 3.7% of healthy controls. This *Science* paper was launched as a breakthrough with important implications for the prevention and treatment of the disorder. Indeed, in some patients antiretroviral treatment was requested for some patients, and the demand for a universal test for XMRV (at a cost of more than US $500 per test) led to increased funding from several patient organizations. Soon after, several concerns about the study were raised. These included consideration of bias, reverse causality, and lack of generalization. The authors of the *Science* paper refuted all claims [4].

Two new papers published in the July issue of *Science* seem to reject a possible role for XMRV in chronic fatigue syndrome. The first study [5] could not find any evidence of XMRV infection in the same samples tested in the 2009 *Science* study. Furthermore, evidence was presented alluding that the Tag polymerase and other commercial laboratory reagents used in the 2009 study were contaminated by viral mouse DNA. The second study [6] showed that XMRV probably arose as the consequence of a recombination event between two mouse proviruses that occurred between 1993 and 1996, many years after the first description of chronic fatigue syndrome.

With these new data, the editor of *Science* approached Lombardi and colleagues to voluntarily retract their paper. The authors declared "it is premature to retract our paper" and thus the editor published an editorial expression of concern attached to the original paper [7].

Despite the enormous pressure to find cause and cures for many common debilitating conditions, such as chronic fatigue syndrome, full scientific proof and validation remain paramount. Nonetheless, the story of XMRV and chronic fatigue syndrome should encourage basic and clinical research to continue at full strength to reconfirm results until the true cause of a disease, such as the one discussed here, is identified.

XMRV = xenotropic murine leukemia virus-related virus

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"When a man is wrapped up in himself he makes a pretty small package"

John Ruskin (1819-1900), British author, art critic, and social reformer

Lymphatic Vessels: Structure and Function

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KEY WORDS: lymphatic capillaries, specialized inter-endothelial junctions, interstitium of connective tissue, immune cells

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Lymphatic vessels are part of the lymphatic system. The vessels evolved phylogenetically only after it became necessary for multicellular organisms to remove fluids and proteins from tissue and return them to the bloodstream [1]. In humans, the lymphatic system begins to develop between the sixth and seventh week of embryonic development, at a time when the cardiovascular system is already functioning [2].

In an article on the drainage function of lymphatic vessels Phylida Brown states that Hippocrates (approximately 400 BC) described vessels bearing "white blood." In 1622, the Italian physician Gasparo Asellius discovered lymphatic vessels in the mesenterium of a fed dog, and he described them as "milk veins." However, the findings of three British anatomists, William Hunter, William Hewson and William Cruikshank, published during the period 1740 to 1787 were to prove hugely influential in lymphatic vessel anatomy. They dubbed lymphatic vessels (lymphatics) "absorbents" (vasa absorbantia), because their function is to absorb liquid waste. Moreover, Hewson already acknowledged the fact that lymphatic vessels evolved to produce a substance called lymph, which includes small particles (now known to be lymphocytes) essential to body growth and health. In 1995, Terence Ryan pointed out that the findings of the above mentioned three anatomists were cited in an edition of *Encyclopaedia Britannica* as early as 1806, and its readers were introduced to the function of lymphatic vessels.

Lymphatic vessels are part of the lymphatic and immune system in the body. Their draining function is very important especially during an inflammation

The 20th century brought significant advances in lymphatic system research, as new findings were published on recirculation of lymphocytes and proteins, on the ultrastructure of lymphatic capillaries, on the spontaneous contractility of lymphatic vessels, and on the transport of microorganisms by the lymph. These discoveries enabled lymphatic vessels to be visualized in vivo using vital stains, contrast substances, and radionuclide lymphangiograms [3].

The anatomic area of the lymphatic system is extensive. Olszewski [1] identified the following to be a part of the lymphatic system: interstitium, lymphatic vessels, lymphatic organs, and their mobile messengers – migrating cells. The lymphatic system functions as one whole, despite being made up of numerous differently arranged lymphatic organs across the entire body, as well as billions of individual, free-moving lymphocytes that circulate in the bloodstream, lymph and interstitial fluids. Lymphatic organs are connected by two vessel systems – the lymphatic vessel system and the blood vessel system [4]. The lymphatic tissue of a young person weighing 70 kg contains approximately 10^{12} lymphocytes, i.e., 1 kg [5]. It must be noted that the lymphatic system is part of the immune system.

Lymphatic vessels form a sort of "second circulatory system" in the body – lymphatic circulation. However, our knowledge is rudimentary when compared to our knowledge of blood vessels. In recent years, scientists in several laboratories began to study lymphatic vessels intensively and found evidence to support the fact that the "second circulatory system" is crucial for the normal functioning of the immune system and that it plays an important role in the pathogenesis of numerous diseases; for example, cancer, lymphedema, asthma, and various inflammatory diseases [6].

Lymphatic vessels form a drainage system in the body, running parallel with veins and collecting lymph from the whole body. The system of lymphatic vessels includes lymphatic capillaries, prenodal lymphatic vessels and postnodal lymphatic vessels, which converge into larger lymphatic vessels to bring lymph into the ductus thoracicus and ductus lymphaticus dexter that lead into the confluences of large veins. In the histological picture, valves similar to those in veins are found in the lumina of lymphatic collector vessels. Their function is to prevent the backwards flow of lymph.

It is hard to differentiate lymphatic vessels – especially lymphatic capillaries – from small blood capillaries in a histological examination of bioptic and especially necrotic human material. This fact has helped lymphatic vessels to escape detection by pathologists, and certainly contributed to the fact that lymphatic vessel research was always second to that of blood vessels over the years. A significant turn came in 1990 with the discovery of molecules that specifically control the development and growth of lymphatic vessels

(lymphangiogenesis), and with the identification of molecules specific for the endothelium of lymphatic capillaries [7].

Of the above mentioned molecules, one of the first was the vascular endothelial growth factor receptor-3 with its ligand VEGF-C [8]. In recent years, scientists explored the possibility of using VEGF-C and VEGF-D growth factors, which are known to cause lymphangiogenesis, in the treatment of tissue edema in various diseases and in diabetic wound healing [9]. Baluk et al. [10] described the effect of growth factors on lymphatic vessels in mice with experimentally induced chronic respiratory tract infection. The inhibition of VEGFR-3 completely prevented the growth of lymphatic vessels but not blood vessels. Insufficient lymphatic vessel growth increased the edema of the mucosa and decreased the hypertrophy of regional lymph nodes. Application of VEGF-C or VEGF-D evoked lymphangiogenesis but did not cause angiogenesis of blood vessels.

Several years ago, Banjeri et al. [11] identified a specific protein on the surface of lymphatic endothelial cells and macrophages and named it LYVE-1. They found it to be a receptor for hyaluronan. This receptor is located in the cell wall of lymphatic vessels, yet it was not found on blood vessels. Using LYVE-1 antibodies, they managed to visualize the endothelium of lymphatic vessels in tissue sections from several organs, including the skin [12]. Another molecule that can be used to identify lymphatic vessels in tissue sections, podoplanine, is a membranous glycoprotein found in endothelial cells of lymphatic capillaries. It has not been found in blood capillaries.

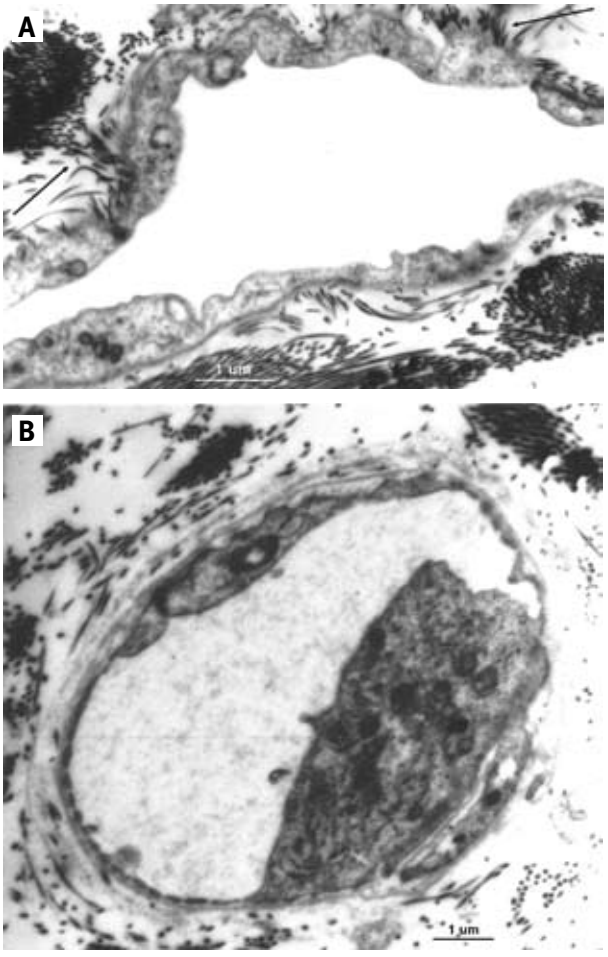
Lymphatic capillaries, often called initial lymphatics, are the thinnest lymphatic vessel. Similar to blood capillaries, lymphatic capillaries are an integral part of connective tissue, especially loose connective tissue [Figure 1].

Both blood capillaries and lymphatic capillaries are crucial to microcirculation in the loose connective tissue. Microcirculation also includes tissue channels that are the morphological substrate of extravascular microcirculation in the loose connective tissue [13]. By means of microcirculation, loose connective tissue facilitates cell nutrition and drainage of metabolism products. It is also the site of inflammatory processes.

The morphological background of microcirculation is formed by blood capillaries, the interstitium of connective tissue, and lymphatic capillaries. Tissue channels are situated in the interstitium [13]. The interstitium consists of the extravascular space between capillary walls and tissue cells. Components of the interstitium include intercellular substances (matrix), tissue fluid and controlling immune cells. The intercellular matrix is made up of fibers (collagen, elastic and reticular fibers) and amorphous substance. The amorphous substance comprises glycosaminoglycans, proteoglycans and glycoproteins. The major part of the amorphous substance is formed by glycosaminoglycan, i.e., hyaluronan. Hyaluronan forms a supporting construction for the migration and adherence of immune cells in connective tissue. Natural hyaluronan is a polymer with high molecular weight of usually over 10^6 D. In the case of an inflammation, the intercellular matrix accumulates hyaluronan fragments with low molecular weight. Hyaluronan is not only a static structural element of the interstitium but is also subject to constant metabolic turnover. During this process, hyaluronan enters lymphatic capillaries and is subsequently transported within the prenodal lymph into regional lymph nodes, where approximately 90% of it

Specialized interendothelial junctions of lymphatic capillaries enable drainage of tissue fluid, immune cells and debris from the interstitium of connective tissue to the lymph nodes

Figure 1. Electron microscopic microphotographs depicting the ultrastructure of the lymphatic capillary **[A]** and blood capillary **[B]**. There are anchoring filaments (arrows) from the adjacent connective tissue attached to the cell membrane of lymphatic capillary endothelium. The blood capillary endothelium is fenestrated and surrounded by a basement membrane.



VEGF = vascular endothelial growth factor receptor

undergoes degradation and the remaining 10% is transported by efferent lymph into the blood circulation to be metabolized later in the liver [14].

It is well known that hyaluronan may also act as a signaling molecule for cells. Interactions between the intercellular matrix and molecules on the cell surface play an important role in cell migration. Cells interact with each other through ubiquitous recognizing molecules called adhesive molecules [15]. Cell migration is crucial for morphogenesis during embryonic development. It plays an important role later in tissue repair and immunological control.

In contrast to erythrocytes, leukocytes act mostly outside the blood flow. Leukocytes exit blood circulation and enter the surrounding interstitial connective tissue where they perform immunological control [Figure 2].

Important functions of leukocytes include the identification of antigens, the destruction of invasive bacteria, and the removal of debris. The migration of leukocytes in the interstitium involves receptors acting as "legs" that help moving cells to adhere to the intercellular matrix or other cells.

Lymphatic vessels play an important role in the homeostasis of extracellular fluid. The average human body weighing 65 kg contains 3 L of blood plasma and 12 L of interstitial fluid. Up to 8–12 L of afferent lymph are produced each day, of which 4–8 L of ultrafiltrate are reabsorbed into the bloodstream in the lymphatic nodes. Lymphatic vessels transport 4 L of efferent lymph into the bloodstream daily. The concentration of proteins in plasma, interstitial fluid, afferent lymph, and efferent lymph is 70 g/L, 20–30 g/L, 20–30 g/L, and 60 g/L, respectively. The fluid turnover (including the volume of fluid reabsorbed in the lymph nodes) reaches up to two-thirds of the total volume of interstitial fluid every 24 hours.

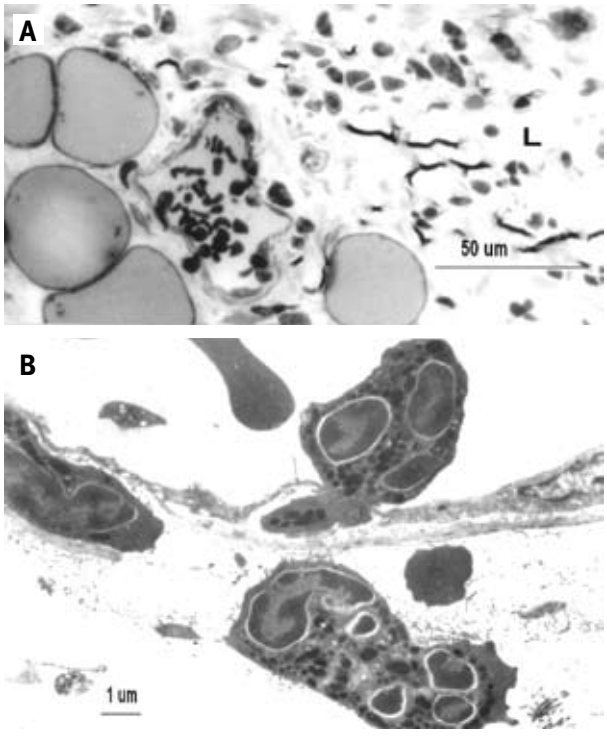
The accumulation of tissue fluid in the interstitium can cause edema in the afflicted area. Edema may occur if microvascular filtration (in the blood capillaries and venules) exceeds the lymphatic drainage for a sufficiently long period. This may be caused by a high rate of filtration or a weak flow of lymph, or a combination of both [16].

In steady state, the extravasation of fluids and proteins from blood vessels is balanced by lymphatic drainage and a return into the bloodstream. The lymphatic system is far more important in achieving homeostasis in tissues than was previously thought. Recently, it was even shown that the skin on the lower extremities contains a denser and more extensive network of lymphatic capillaries than the skin of the upper extremities [17]. Due to orthostasis, the lower extremities have a higher filtration pressure and a higher influx of fluids. Those authors [17] state that the capacity for lymph transport in the lower extremities is greater in order to compensate the higher influx of interstitial fluid caused by the effects of orthostasis and gravity.

As early as the turn of the 20th century, Starling [18] successfully demonstrated that lymphatic vessels play an important role in the regulation of hydrostatic and oncocytic pressure in the interstitium [18]. Plasmatic proteins that had entered the extravascular area (the interstitium) may return to the blood in two ways: a fraction will return through the cell wall of blood vessels, while the greater part will reach the lymphatic capillaries and will be led by the system of lymphatic vessels around and back into the bloodstream. A dynamic equilibrium is normally reached between filtration, reabsorbtion into blood capillaries, and extra tissue fluid drainage (clearance) into lymphatic capillaries, which is why no edema occurs

Edematous fluid containing more than 10 g/L of proteins is considered to be high protein fluid and signals the inflammatory origin of such edema. Plasma proteins are taken up from the interstitial tissue by lymphatic capillaries as well as by proteolysis – mainly in cases of high protein edema [19]. Proteolysis is facilitated by multiple types of cells, of which macrophages are the most important. While macrophages remain important for inflammation, we should not forget that they are present in healthy tissue as well. It has been found that 1 cm^3 of loose connective tissue contains approximately 10^7 macrophages. Their amount increases during the inflammatory process (almost tenfold). Földi and Casley-Smith [19]

Figure 2. Microphotograph **[A]** and electron microscopic microphotograph **[B]** showing the diapedesis of leukocytes – neutrophilic granulocytes – through the wall of postcapillary venule. The leukocytes are visualized passing from the venular lumen into the interstitium. The microphotograph also visualized the lymphatic capillary (L) near the venule, and a lymphocyte in its lumen.



highlighted the role of macrophages in the development and retention of chronic inflammation. Activated macrophages produce chemokines. Macrophages also play an active role in phagocytosis and cleave proteins. Lymphologists presume that tissue proteolysis is a physiological process that provides building material for tissue cells. They believe that proteolysis in tissues plays an important role in the inflammatory process.

However, lymphatic vessels are not simply passive drainage tubes draining interstitial tissue fluid. Spontaneous contractility of lymph vessels is utilized in lymph transport. Active contractions of lymph vessels were described by Hewson as early as 1774, but their important role was recognized only recently. Regular contractions of lymph vessels at a frequency of 2–4 per minute were observed in vitro in lymphatic vessels isolated from cattle mesenterium. Lymphatic vessels were even found to be able to pump fluid against the hydrostatic gradient. Spontaneous contractility of prenodal lymphatic vessels has been observed in humans, and these contractions were demonstrated to be driving the lymph [20]. Therefore, the contractility of lymphatic vessels is seen as an important driving force of lymph propulsion. The lymph flow is controlled by neuroendocrine mechanisms. Catecholamines have been proved to promote the contractility of lymphatic vessels and foster lymph flow both in vitro and in vivo. Both adrenergic and cholinergic nerves were detected in lymphatic vessel walls.

Another physiological function of the lymph vessel system includes the transport of blood cells. The peripheral (afferent, prenodal) lymph contains rare erythrocytes from the interstitium. Tissue fluid that had not been absorbed into blood vessels is drained away through lymphatic vessels, together with proteins, macromolecules and cells that become part of the lymph upon their entry into lymphatic capillaries. Also, small lymphocytes, plasma cells, macrophages, monocytes, neutrophilic and eosinophilic granulocytes, and large basophilic cells were all found in the prenodal lymph cannulated from lymphatic vessels from limbs and some organs of sheep [21]. The afferent lymph is populated mainly by monocytes, because they continuously exit the bloodstream, migrate, undergo differentiation in the interstitium and perform their function, and enter the lymphatic capillaries [21]. Monocytes, macrophages and dendritic cells are usually not found in the efferent lymph [22].

The existence of lymphatic capillaries influences lymphocyte recirculation and immune cell movement through the interstitium of connective tissue in peripheral organs (e.g., intestine, skin, kidney and others)

The central (efferent, postnodal) lymph contains noticeably more blood cells, because lymph nodes are where flowing lymph receives lymphocytes from postcapillary venules. This process was described by Gowans and Knight in 1964 [23]. Through the postnodal lymph, lymphocytes return to the bloodstream; this is called lymphocyte physiological recirculation. Those researchers [23] described a modified endothelium in the postcapillary venules of the lymph nodes which gives these cells a specific appearance under the microscope, and accordingly named them high endothelial venules. The specialized endothelium of postcapillary venules in the lymph nodes enables subpopulations of lymphocytes to move from the bloodstream into the lymph more readily than would be the case for any other tissue [4]. The extravasation of lymphocytes begins with the interaction between lymphocytes and high endothelial venules, which is in turn made possible by the specific interaction between the receptor and the ligand. Morgan and Holt [24] found that only lively, completely functional lymphocytes enter the lymph nodes from the bloodstream, having a normal cell surface capable of interaction with the endothelium of high endothelial venules.

It was later found that lymphocytes also continuously migrate (recirculate) from the bloodstream into the lymph in the intestine, through lymphatic tissue called gut-associated lymphoid tissue [25]. By cannulating the lymph from afferent lymphatic vessels in sheep, it was found that lymphocytes also recirculate in the skin, kidney and other organs. Recirculating lymphocytes carry out immune control in almost all tissues, and they are responsible for spreading immune responses and distributing immune memory in the entire organism [26]. In regulating immune responses the important role of vitamin D was stressed by Toubi and Shoenfeld [27].

The majority of mature lymphocytes continuously recirculate from the blood into tissues, and back again into the bloodstream through lymph at a rate of once or twice a day. The 12–24 hour cycle of recirculation is repeated again until the cell finds its antigene, or dies [28].

Postcapillary venules that resemble high endothelial venules in the lymph node paracortex are also found in tissue afflicted by chronic inflammatory processes. These vessels are often surrounded by a large number of lymphocytes [29]. It is known that a large number of adhesive molecules, cytokines and chemokines take part in the migration of immune cells from the bloodstream into tissues [28]. However, only a few experiments aimed at studying the mechanisms of immune system cell entry from interstitial connective tissue into afferent lymphatic vessels have been carried out [22]. Irjala and co-authors [30] identified a molecule which they named the "common lymphatic endothelial and vascular endothelial receptor-1" (CLEVER-1) that mediates the bonds of lymphocytes to both high endothelial venules and lymphatic vessels. The authors suggest that CLEVER-1 regulates the recirculation of lymphocytes and is active in the migration of leukocytes to the sites of inflammation. It was recently found that the exit of T lymphocytes from

CLEVER-1 = common lymphatic endothelial and vascular endothelial receptor-1

peripheral tissues into lymphatic vessels is dependent on the chemokine receptor CCR7 [31].

The migration of subpopulations of small lymphocytes is tissue-specific. Naïve lymphocytes are programmed to recirculate through the lymph nodes. In contrast to naïve lymphocytes, memory and effector lymphocytes exit the blood and migrate through loose connective tissue situated in the peripheral organs, e.g., in the intestinal mucosa, lung interstitium, skin, or joints [28].

In studying the lymphatic system during the ontogenetic development of sheep, Cahill and team [5] found extensive recirculation of T lymphocytes and dendritic cells through peripheral tissues. The authors concluded that the considerable recirculation of T lymphocytes is the same feature of the fetal immune system as liveborn animals.

By cannulating lymphatic vessels, lymphologists found in as early as 1980 that lymphocyte migration differs considerably from the migration of other cells from the bloodstream. Lymphocytes migrate from the bloodstream into lymph even if an exogenous antigene is absent. Lymphatic capillaries are entered by antigens from interstitial connective tissue. It may be said that almost all natural immune system stimulation is caused by the entry of an antigene (e.g., viruses, bacteria, allergens) through intact or damaged skin or mucosa. As soon as antigens enter the interstitium in connective tissue, they quickly enter the lymph through specialized interendothelial junctions in the walls of lymphatic capillaries. Afferent lymphatic vessels then deliver the antigens into the regional lymph node in which the immune response is induced. This response is then "sent" into the entire body through lymphatic and blood circulation [4]. The study of the contents of efferent lymph taken from cannulated efferent lymphatic vessels of experimental animals has shown that up to 5 ml of lymph per hour and 30–50 million lymphocytes per hour can be taken from one lymph node at ease, weighing approximately 1 g. Approximately 90% of small lymphocytes in lymph flowing from the node had entered it from the blood through high endothelial venules, 2–3% were proliferated in the lymph node, and 5–10% arose from the peripheral (afferent) lymph. The afferent lymph contains 10–20% macrophages and some small lymphocytes, while the efferent lymph contains almost no macrophages but 20–30% of small lymphocytes. The fate of macrophages that enter the lymph node from the afferent lymph is not clear, but about 10^7–10^8 macrophages will vanish completely every day in a lymph node weighing 1 g [4]. A lymph node in which an immune response is taking place shows changes in cell migration. The migration of lymphocytes from the bloodstream to the lymph increases.

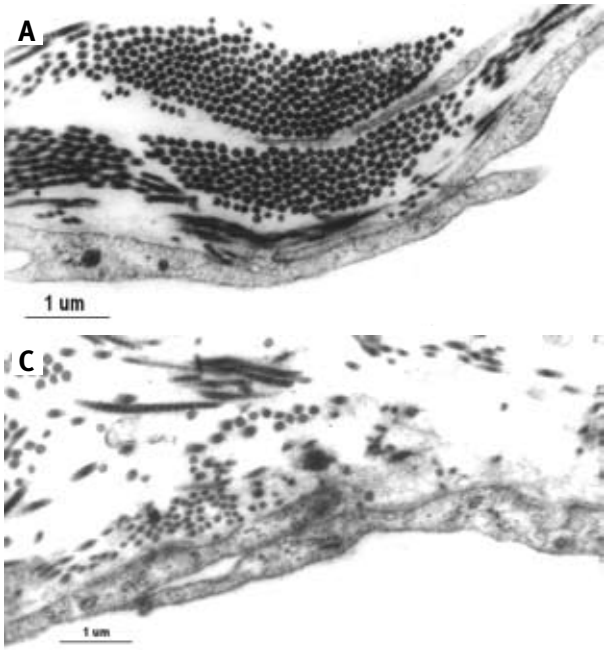
Afferent lymphatic vessels enable the transport of other cells of the immune system, such as dendritic cells, from peripheral organs (skin, synovial membrane, and others) to the regional lymph nodes. Dendritic (antigen-presenting) cells meet foreign antigens in the skin. However, lymph nodes are the optimal place for presenting antigens to T lymphocytes; therefore, it is necessary for dendritic cells to enter the lymphatic capillaries and travel to the lymph nodes in the prenodal lymph. At present, we know that dendritic cells are attracted to the lymphatic capillaries (initial lymphatic vessels) by the CCL21 and CCR7 chemokines [32].

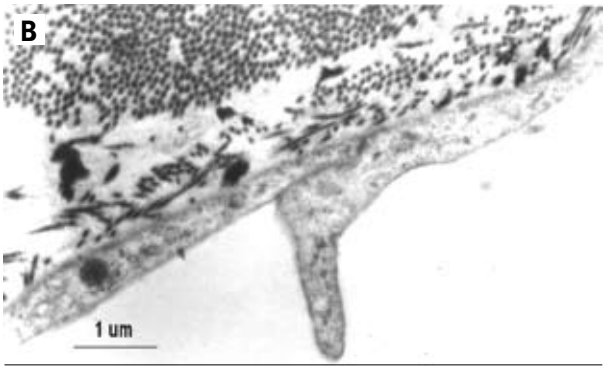
The drainage function of lymphatic vessels is crucial for inflammatory reactions in the interstitium. During an inflammation, lymphatic vessels even proliferate, and lymphangiogenesis occurs. This process was described in 1937 by Pullinger and Florey [33], who stressed that debris is removed from the place affected by inflammation by the lymphatic vessels, either directly or through phagocytic cells. Olszewski [34] reported having found cell debris in the afferent lymph obtained by cannulating superficial lymphatic vessels in lower extremities of humans. The afferent lymph contained apoptotic cells. He described apoptosis in 20% of lymphocytes in the afferent lymph. In addition, he observed fragments of membranes, nuclei, mitochondria and fibrinogen in human afferent lymph by electron microscopy. Macrophages and cell debris were also reported to have been found in the lymphatic capillaries of the synovial membrane obtained from operation material from synovectomies in patients with rheumatoid arthritis and juvenile idiopathic arthritis [35,36]. The observation of lymphocytes, monocytes, macrophages and cell debris in the lumina of some lymphatic capillaries confirmed the drainage function of lymphatic vessels in the synovial membrane.

The system of lymphatic vessels forms a functional entity with the pre-lymphatic tissue channels situated in the interstitium of connective tissue. Using electron microscopy, these tissue channels were described by Casley-Smith [37]. The results of microscopic observations have suggested that the most peripheral part of the lymphatic system is a completely open system of tissue channels. The traditional concept of the blind ending (or beginning) of lymphatic vessels is seen by lymphologists to be a result of the retrograde filling methods used in morphology.

In conclusion, we would like to describe the histological structure of the most delicate lymphatic vessels – lymphatic capillaries, and answer the following question: How do the immune cells that perform immunological control in connective tissue enter the lymphatic capillaries from the interstitium? The walls of lymphatic capillaries are composed of endothelial cells. Lymphatic capillaries are not lined by a basement membrane. The surrounding connective tissue fibers are directly anchored to the endothelial cells of lymphatic capillaries [Figure 1]. This fact was recognized by Pullinger and Florey in 1935 using a light microscope to study the skin of experimental animals with edema [38]. Endothelial cells of lymphatic capillaries are interlinked with intercellular junctions, the details of which were revealed by the method of

Figure 3. Electron microscopic microphotographs **[A,B,C]** depicting the ultrastructure of specialized intercellular junctions between the endothelial cells of lymphatic capillaries. Specialized interendothelial junctions consist of overlapping extensions of adjacent endothelial cells. Bundles of collagen fibers and elastic fibers are depicted in the surrounding connective tissue.

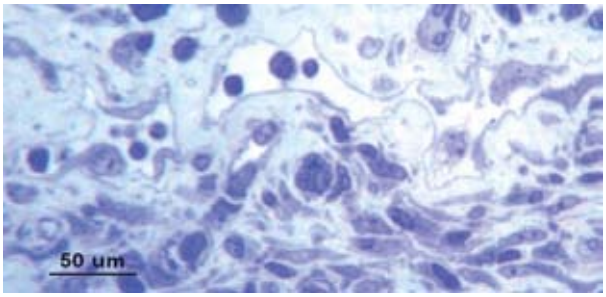




transmission electron microscopy. As described by Leak and Burke in 1996 [39], specialized inter-endothelial junctions play a substantial role in the draining function of lymphatic capillaries. These specialized junctions consist of mutually overlapping endothelial extensions. Cell membranes of overlapping endothelial extensions are not connected by intercellular adhesive junctions. The adjacent connective tissue fibers (anchoring filaments) are anchored only to the external extension. While the external extension of the endothelial cell is firmly attached to the adjacent connective tissue by anchoring filaments, the internal extension (flap), which is unattached, may act as a single valve. When the interstitial pressure rises, the internal extension bends into the lumen of the lymphatic capillary, creating a direct communication between the interstitium space and the lymphatic capillary. As soon as the pressure in the lymphatic capillary lumen exceeds the pressure in the adjacent tissue, the internal extension will cover up the external extension [Figure 3]. This mechanism guarantees a one-way flow to transport interstitial fluid, large molecules and cells from the interstitium into the lumen of the lymphatic capillary. Specialized inter-endothelial junctions may open up as much as several micrometers [40]. As the specialized inter-endothelial junctions are similar to valves both in their morphology and function, they were later named endothelial microvalves or primary valves.

Lymphatic capillaries are part of the microcirculation in the connective tissue. Immune cells that perform immunological control in peripheral organs enter the lymphatic capillaries from the interstitium [Figure 4].

Figure 4. Microphotograph depicting lymphatic capillaries. Their lumina contain several lymphocytes and macrophages (semithin resin section stained with toluidine blue).



These cells are then further transported in lymph by prenodal lymphatic vessels into lymph nodes, while some of them are subject to recirculation into the blood circulation.

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Capsule

New targets for intervention in the treatment of postmenopausal osteoporosis

Postmenopausal osteoporosis is a disease of high bone remodeling, with an imbalance of bone resorption over bone formation, resulting in decreased bone mineral density and disruption of bone microarchitecture. With our improved understanding of the molecular and cellular regulators and mediators of bone remodeling, new targets for therapeutic intervention have been identified. Lewiecki reviewed the new approaches. Receptor activator of nuclear factor κB ligand (RANKL) is the principal regulator of osteoclast differentiation, activity and survival; denosumab, a fully human monoclonal antibody to RANKL, inhibits bone resorption and is approved for the treatment of women with postmenopausal osteoporosis at high risk of fractures. Cathepsin K is a protease produced by activated osteoclasts that degrades the protein matrix of bone. An inhibitor of cathepsin K, odanacatib, is in phase III clinical trials for the treatment of postmenopausal osteoporosis; it decreases bone resorption while seeming to suppress bone formation less than other antiresorptive agents. Sclerostin is a cytokine produced by osteocytes that inhibits osteoblastic bone formation; investigational monoclonal antibodies to sclerostin, such as AMG 785, have osteoanabolic properties with the potential to improve clinical outcomes in patients with osteoporosis. These and other novel interventions that target newly recognized regulators of bone remodeling are promising agents for the treatment of osteoporosis.

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Eitan Israeli

Understanding Fibromyalgia and its Resultant Disability

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KEY WORDS: fibromyalgia, central sensitization, disability, sleep disturbance, pain

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The introduction of the American College of Rheumatology fibromyalgia classification criteria 20 years ago heralded two decades of professional acceptance and enhanced multidisciplinary research in the pathogenesis and therapy of fibromyalgia [1]. Whereas the initial criteria included tenderness on pressure (tender points) in at least 11 of 18 defined anatomic sites with the presence of widespread pain, in the 2010 set of proposed criteria it is clear that apart from the pain other seminal features of the disorder – namely cognitive dysfunction, unrefreshing sleep, fatigue and mood disorders – play an important role in the diagnosis [2]. Many journals today dedicate scientific papers to the pathogenesis and treatment of fibromyalgia, and grants are provided worldwide to investigate the disorder.

The leading symptoms limiting vocational tasks in patients with fibromyalgia are pain, tiredness, muscle weakness, and memory and concentration difficulties

Fibromyalgia is a chronic manifestation of diffuse musculoskeletal pain that is more commonly encountered in women (9:1 female:male ratio) and is present in all ethnic groups, climates and cultures [3-7]. In the following review we will explore the emergence of the fibromyalgia syndrome and its implications with regard to disability of disability.

PATHOGENETIC ASPECTS OF FIBROMYALGIA

Although a clear sequence of events to explain the clinical manifestations of fibromyalgia is lacking, many advances have been made in unraveling its pathogenesis. Firstly, a deficiency of serotonin and a surplus of substance P have been recorded in the cerebrospinal fluid of patients with fibromyalgia [8]. Serotonin deficiency may be related to the altered sleep patterns, especially during stage 4 sleep (deep sleep) in whose initiation serotonin plays a major role [9]. On the other hand, a surfeit of substance P, a brain and spinal cord neuropeptide released from the terminals of specific sensory nerves, plays a role in pain signaling, integration and modulation, suggesting that fibromyalgia patients have an enhanced sensitivity to pain.

In addition, central sensitization is another mechanism to explain this increased perception of pain in fibromyalgia patients. Central sensitization includes spontaneous nerve activity, expanded receptive fields (resulting in a larger geographic distribution of pain), and augmented stimulus responses within the spinal cord [10]. This pathway involves triggering an N-Methyl-D-aspartate (NMDA) receptor, which is thought to be involved in this abnormal temporal summation of pain stimuli [11].

Muscular pathology has also been implicated in the pathophysiology of tender points in the fibromyalgia syndrome. Decreased growth hormone concentration, which is essential for muscle function, may explain the extended muscle pain seen after exercise in fibromyalgia patients [12]. More convincing arguments have been published regarding abnormal pain amplification at the level of the spine as the likely mechanism for increased pain in fibromyalgia patients [13].

It is currently recognized that familial aggregation is often encountered in fibromyalgia. Hudson et al. [14] reported that the odds ratio for fibromyalgia in a relative of a fibromyalgia proband versus a relative of a rheumatoid arthritis proband was 8.5. Learned patterns of behavior probably portray certain families. Several candidate genes have been suggested to mediate this association. Early research into the genetic basis of fibromyalgia was directed towards the possibility of linkage to human leukocyte antigens. Burda and collaborators [15] reported that the HLA1 DR4 antigen was detected in 64% of patients with fibromyalgia versus 30% of healthy controls. Several teams observed a higher frequency of the S/S genotype of the serotonin transporter gene (*5-HTT*) promoter region in fibromyalgia patients compared to healthy controls. An increased frequency of the *5-HTT* gene was demonstrated among patients versus controls [16]. Other studies focusing on the serotonin receptor subunit genes *HTR3A* and *HTR3B* failed to exhibit any polymorphism among fibromyalgia patients [17].

Several inciting factors have been shown to trigger the emergence of fibromyalgia. Some studies show that infections

such as hepatitis B virus, hepatitis C virus, human immunodeficiency virus and Lyme disease triggered fibromyalgia [18]. Past history of negative life events has often been described among patients with fibromyalgia, and increased rates of post-traumatic stress disorder associated with childhood abuse, trauma or anxiety have been reported [16,19,20]. Many fibromyalgia patients have psychological disorders that have further challenged its validity: Is fibromyalgia a reflection of a psychiatric illness or an illness on its own? Although approximately 30%–50% have anxiety, depression, somatization or hypochondriasis, many fibromyalgia patients do not have psychiatric comorbidities [21].

DISABILITY IN FIBROMYALGIA

Chronic pain conditions are the most common reason for disability leave from work. In addition, these conditions account for the highest indirect costs for society and also account for an individual economic, social, educational and vocational burden [22]. Musculoskeletal disorders and gender interact in a way that increases sick-leave rates.

In Sweden in 2001, women accounted for 58% of costs resulting from sickness absence from work [22]. Excluding pregnant women, the sick-leave rate was 25% higher than in men [23]. In an 11 year prospective cohort study of people with spinal and shoulder pain, 27% of the women and 14% of the men had been granted a disability pension. Interestingly, the leading causes that were related to approval of disability pensions were foreign citizenship, gender, and number of sick-leave days per spell [24].

Vocational rehabilitation ameliorates depressive symptoms and improves functional ability, although pain symptoms do not disappear

The most frequently mentioned symptoms that affected vocational activities were found to be pain, sleep disturbances, and difficulties in performing motor tasks. The leading symptoms limiting vocational tasks in patients with fibromyalgia were found to be pain (87%), tiredness (80%), muscle weakness (73%), and memory and concentration problems (51%) [25].

This economic burden often affects fibromyalgia individuals, particularly those of lower socioeconomic status. These individuals are more often dependent on the government health care system, which has not accounted for the complexity and implications of this syndrome sufficiently. In fact, fibromyalgia is more prevalent in the lower socioeconomic status population perhaps due to other confounding variables. First, this population tends to work in more manual labor jobs, which may facilitate more pain and injuries. Second, this population tends to be overweight – another risk factor for pain; and third, this population tends to do more household work, in general and because they are less likely to afford housekeeping services, an additional risk factor for pain [26]. Other reports have also showed an inverse relationship between chronic pain and educational level [27].

Children with fibromyalgia are usually diagnosed months to years following the initial manifestations; the precious time lost until proper diagnosis is made is characterized by social isolation and malfunction and loss of days at school, which directly contribute to a lower educational level in adulthood [28]. Sleep disturbances also greatly affect how well children pay attention in school and even their overall attendance in school. In addition, lack of sleep may lead to muscle deconditioning, which leads to loss of muscle strength particularly in women. Kilbom [29] suggested that the differences in body size, muscle strength, oxygen uptake and hormones contribute to musculoskeletal disorders. This muscle deterioration may even perpetuate musculoskeletal pain but, as discussed earlier, is less convincing than the central sensitization theory of pain in these individuals. Physicians and caregivers should recognize these problems and try to promote full attendance at school or, if the implications are severe enough, consider homeschooling. The impaired learning may have lasting effects on their ability to succeed in future workplaces in addition to their inability to attain jobs in a higher echelon.

Therefore, fibromyalgia patients may experience a vocational and socioeconomic vicious cycle. Fibromyalgia symptoms such as sleepiness and inability to concentrate may affect individuals as early as childhood, which may result in poor academic performance, limiting these individuals to lower echelon jobs, mainly manual labor. Manual labor jobs may then further disable these patients at the workplace.

CURRENT IMPLICATIONS FOR DISABILITY

Now that fibromyalgia is gaining general acceptance in the medical community, many patients are applying for modifications at their workplace to relieve these burdens. Several recent reports show that 34%–77% of women with fibromyalgia have succeeded in preserving their jobs [22,30-32]. Those who continued working tended to be older, had fewer difficulties in daily activities and lower severity of symptoms, especially pain [27]. The needs of fibromyalgia patients should be understood, and the importance of identifying necessary individual adjustments should be considered to keep these women working and to lessen the economic, social and vocational burdens on themselves, their family and society. Possible explanations may be that although these individuals are applying for modifications, employers, physicians and other health care administrators are not familiar with this syndrome or with the required modifications that are necessary to keep women at work. This fact, in addition to other social and economic burdens, may contribute to them leaving work. Therefore, it is recommended that younger patients be provided with the appropriate aid and setting for their disability at work, to enable them to continue working [22,30-32].

Although previous studies have shown that patients who were diagnosed with fibromyalgia have not sought medical care since they are apprehensive and reluctant to believe that their syndrome is real, recent studies show otherwise [33]. In a study conducted in the Negev district in southern Israel, fibromyalgia patients were compared with hypertensive and diabetic patients regarding the costs for hospitalization, day care, specialists and diagnostic services. The study concluded that although the hospitalization costs were similar for the three chronic diseases, the need for specialists and diagnostic services was statistically significantly greater among patients with fibromyalgia in comparison to the other two diseases [34]. Perhaps this statistic may be elucidated by the fact that only 55% of primary care providers in that region were familiar with the fibromyalgia syndrome [3]. These studies underlined the significant burden that the fibromyalgia syndrome casts on the health care budget. It was indicated that patients spend much time with specialists and undergoing unnecessary diagnostic procedures, which impose a significant economic burden in addition to the time taken off from work, costs of transportation to the medical services, and diagnostic studies that are not always completely covered by their medical insurance [3,34]. Furthermore, specialists who evaluate the necessity of worker's compensation for these patients are essential since physicians are usually not able to assess these needs.

Flexibility at work, allowing those who work full time to take a break mid-day as well as short breaks can significantly reduce the number of fibromyalgia patients leaving their jobs

In a study analyzing the way 23 assessment centers in the United States evaluated chronic pain patients at the workplace, it was found that these assessors focused mainly on the physical factors of work performance rather than the individuals as a whole, the specific work demands, the environmental conditions, and how their disorder was influenced by their ability to work [35]. A study in Finland demonstrated that a specific multidisciplinary fibromyalgia rehabilitation program was not superior to a non-specific musculoskeletal multidisciplinary rehabilitation program, further supporting the uncertainty of the type of multidisciplinary programs needed while at the same time supporting the need for them [36]. However, another study, where non-intensive (six sessions, one weekly) multidisciplinary treatment was given to 94 patients who were on the verge of not returning to work or filing for disability pension and were followed at discharge and 6 and 12 months later, showed that although pain symptoms did not disappear, depressive symptoms subsided and functional ability improved [37]. Additionally, another study showed that individuals who had more negative expectations about pain progression and a high perceived functional disability were least likely to return to work after a multidisciplinary treatment program [38].

Many fibromyalgia patients value their work role and it is therefore crucial to assess ways to rehabilitate them and generate solutions so that these individuals will be able to preserve their positions at work [22]. Although rehabilitation programs in the future may help resolve the disabilities these patients experience at the workplace, adjustments should be made now. Firstly, it has been found that various types of work situations involving heavy physical tasks, working above shoulder height, using power grip, and dynamic repetitive tasks should be avoided [22]. Secondly, flexibility at work, including replacing certain tasks and allowing those who work full time to take a break at mid-day as well as short breaks can significantly reduce the number of fibromyalgia patients who leave their jobs. Moreover, flexibility in adjusting working hours day-by-day may help women with fibromyalgia to remain at work. Lastly, a positive social environment where the supervisors and colleagues appreciate the patient was found to be integral in keeping these women working [22].

Fibromyalgia patients have been struggling for years to have their disability acknowledged by the medical community, as have scholars of disability studies. These individuals have been hindered socially and economically by misconceptions held by the medical, social and general communities regarding their clinical condition. These misunderstandings have led to negative attitudes towards rehabilitation programs and compensations for these individuals in the workplace [22]. However, these programs are essential to battle the long-term pain, fatigue and associated symptoms that will lead to increased disability pension for fibromyalgia patients.

In order to keep patients with fibromyalgia at work it is crucial to change current views and to seek and establish constructive strategies for employers as well as medical personnel in order to keep these patients fit for work.

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Capsule

Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells

Exercise, obesity and type 2 diabetes are associated with elevated plasma concentrations of interleukin-6 (IL-6). Glucagon-like peptide-1 (GLP-1) is a hormone that induces insulin secretion. Ellingsgaard and co-workers show that administration of IL-6 or elevated IL-6 concentrations in response to exercise stimulates GLP-1 secretion from intestinal L cells and pancreatic alpha cells, improving insulin secretion and glycemia. IL-6 increased GLP-1 production from alpha cells through increased proglucagon (which is encoded by GCG) and prohormone convertase 1/3 expression. In models of type 2 diabetes, the beneficial effects of IL-6 were maintained, and IL-6 neutralization resulted in further elevation of glycemia and reduced pancreatic GLP-1. Hence, IL-6 mediates crosstalk between insulin-sensitive tissues, intestinal L cells and pancreatic islets to adapt to changes in insulin demand. This previously unidentified endocrine loop implicates IL-6 in the regulation of insulin secretion and suggests that drugs modulating this loop may be useful in type 2 diabetes.

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Eitan Israeli

"Be the master of your will and the slave of your conscience"

Hasidic saying

Cardiogenic Shock in an Elderly Woman: A Diagnostic and Therapeutic Challenge

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KEY WORDS: fulminant myocarditis, giant cell myocarditis, ischemic heart disease, cardiogenic shock

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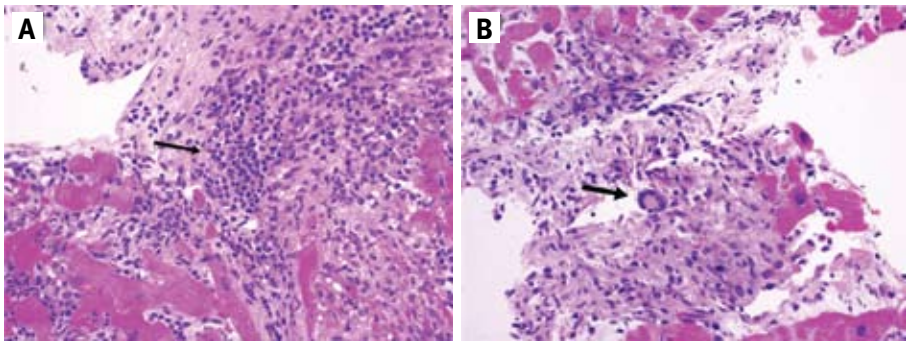
The diagnosis and treatment of acute heart failure and cardiogenic shock as the initial presentation of acute fulminant myocarditis can be very challenging, particularly in elderly patients with previous ischemic heart disease. We present such a case and discuss the diagnostic dilemma and the appropriate diagnostic and treatment strategies.

PATIENT DESCRIPTION

A 76 year old woman was admitted following a few days of excertional dyspnea, chest discomfort, fatigue and a short-lasting fever. Two weeks earlier she experienced right facial weakness followed by ptosis of the right eyelid which gradually subsided. Her medical history included an anterior myocardial infarction in 1993 that was treated with streptokinase with reperfusion. Due to angina she underwent a coronary angiogram in 2000 that demonstrated a long diffuse atheroma in the mid-left anterior descending artery with near complete occlusion and good collateral flow. She was treated conservatively. Echocardiography at the time demonstrated preserved left ventricular function with apical hypokinesis. She was physically active with good functional capacity until the present admission. She also suffered from well-controlled hypertension, mild diabetes mellitus, hyperlipidemia and chronic atrial fibrillation. Her medications included atenonol 50 mg twice daily, aspirin 100 mg hydrochlorozide 25 mg, simvastatin 80 mg, twice daily and coumadin. On physical examination she was afebrile, had mild dyspnea, blood pressure of 111/75 mmHg and irregular pulse of 102/min. Oxygen saturation on room air was 92%. She had distant irregular heart sounds, her lungs were clear to auscultation, and the extremities were without edema. Electrocardiogram revealed atrial fibrillation, right bundle branch block, Q waves and ST-T changes in the inferior leads. The electrocardiogram was not significantly different from a previous ECG. Troponin-T was elevated to 5.5 ng/ml (normal < 0.1) and creatine phosphokinase was 346 U/L (normal < 170). Lactate dehydrogenase reached 1525 U/L (normal < 420), C-reactive protein was elevated to 13.5 mg/dl (normal < 1); the white blood count was normal.

On admission, she developed cardiogenic shock with severe dyspnea, reduced blood pressure and congestion on X-ray. Troponin-T rose to 7.5 ng/ml. Aggressive treatment with an intraaortic balloon pump, inotropes and intravenous diuretics stabilized her condition. Echocardiography revealed severe biventricular failure, diffusely decreased left ventricular contraction with significant mitral regurgitation and no pulmonary hypertension. Mitral regurgitation was not due to a flail leaflet or a ruptured chord. Left and right coronary catheterization revealed no significant change in coronary anatomy from a previous examination with the same severe stenosis in the mid-left anterior descending artery. There was no pulmonary hypertension and no pulmonary artery branch cutoffs. Cardiac index was measured at 1.5 L/min/m^2. Endomyocardial biopsy was performed [Figure]. The biopsy demonstrated severe diffuse myocyte necrosis, a predominantly



Histological specimen (hematoxylin & eosin staining) from the right ventricle demonstrating severe, diffuse necrotizing lymphocytic myocarditis. **[A]** Areas of diffuse myocardial necrosis with large infiltrates of lymphocytes (arrow). **[B]** Severe myocardial necrosis and a nucleated giant cell can be seen (arrow)

lymphocytic infiltrate with sparse giant cells. She was treated with an angiotensin-converting enzyme inhibitor, beta blockers and furosemide. The patient was weaned from the intraaortic balloon pump after 3 days. Repeat echocardiogram 5 days later demonstrated significant improvement in left ventricular function. A comprehensive immunological and infectious workup was negative. The pathology diagnosis was severe, diffuse necrotizing lymphocytic myocarditis. Follow-up 16 months after the event revealed the patient to be free of heart failure, with preserved left ventricular function on echocardiography.

COMMENT

The patient presented with an acute event and it was not clear if the patient was suffering from an acute coronary syndrome or from acute myocarditis. Since the patient was an elderly woman with significant risk factors and a history of ischemic heart disease, the diagnosis of acute coronary syndrome versus myocarditis can be difficult. While myocarditis occurs more frequently in young people, it is not uncommon in the elderly. The diagnosis is often dismissed or overlooked in the elderly due to coexistent coronary disease. Inflammatory indices as well as echocardiography can be helpful in making the diagnosis; however, they are not always conclusive. Even performing a coronary angiogram may not give a definite diagnosis, particularly in a patient with previous complex coronary disease, as in our patient. Fortunately, in the present case we could compare the coronary anatomy to a previous angiogram and conclude that there was no change, suggesting that the coronary lesion was most probably not the cause of the present insult. Indeed, the diagnosis of myocarditis versus ischemia frequently necessitates a myocardial biopsy to prove or disprove the diagnosis. Unexplained, new-onset heart failure of less than 2 weeks duration associated with hemodynamic compromise is a class I indication for endomyocardial biopsy according to recent American Heart Association/American College of Cardiology/European Society of Cardiology guidelines and was indicated in the present case. Even a myocardial biopsy may be inconclusive due to the patchy nature of myocarditis and the considerable sampling error associated with establishing the diagnosis of myocarditis. The use of magnetic resonance T2 imaging and early gadolinium contrast enhancement as well as late enhancement that detects small areas of myocardial necrosis associated with active myocarditis may increase the diagnostic yield of endomyocardial biopsy by guiding the site of tissue sampling [1]. Recent data suggest a promising role for MRI in discriminating myocarditis from myocardial infarction [2]. MRI can demonstrate evidence of acute active inflammation by increased T2 imaging signal, while infarction or fibrosis can be detected by late gadolinium enhancement.

Our patient rapidly deteriorated into cardiogenic shock. This is a classical presentation of fulminant myocarditis: patients are critically ill, with acute severe left ventricular dysfunction and hemodynamic instability. However, these patients have a good long-term outcome [3]. This differs significantly from patients with acute or subacute myocarditis who are initially less ill but have a progressive course that leads to death or the need for cardiac transplantation. Since the prognosis of patients with fulminant myocarditis is excellent if they survive the initial insult, these patients should be treated aggressively in the intensive care unit [3]. They should receive full hemodynamic support including vasopressors and mechanical support by an intraaortic balloon pump or a left ventricular assist device. Standard heart failure therapy should also be started as soon as possible. However, immunosuppressive therapy is not indicated in patients with fulminant myocarditis as this was not shown in the Myocarditis Treatment Trial to improve prognosis [4].

Another acute myocardial inflammatory disease that can resemble fulminant myocarditis is giant cell myocarditis. Although rare, this disease is rapidly progressive and leads to progressive congestive heart failure, frequently associated with refractory ventricular arrhythmia and conduction disturbances. Patients with this disease frequently have other autoimmune diseases. The diagnosis is based on identifying the typical nucleated giant cells on myocardial biopsy in addition to lymphocytes, histiocytes and eosinophils. This disease, in contrast to fulminant myocarditis, carries a poor prognosis. Treatment should consist of steroids with immunosuppressive therapy including cyclosporine and/or azathioprine that significantly improves prognosis [5]. Transplantation can also be an alternative despite the possibility of recurrence in the transplanted heart.

Our main therapeutic challenge in the present case was the possibility of giant cell myocarditis and whether to initiate immunosuppressive therapy. The fact that only a few giant cells were found without eosinophils and histiocytes in the biopsy, together with the rapid clinical improvement with supportive therapy, led us to the conclusion that we are dealing with a case of fulminant lymphocytic myocarditis and not giant cell myocarditis. The patient's immediate and long-term clinical course also supported this diagnosis.

While acute heart failure in myocarditis can be a life-threatening event and the diagnosis can be elusive, timely action with rapid diagnostic tests including a myocardial biopsy and rapid aggressive treatment can help improve the outcome of patients with this disease.

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Capsule

Epidermal growth factor receptor promotes glomerular injury and renal failure in rapidly progressive crescentic glomerulonephritis

Rapidly progressive glomerulonephritis (RPGN) is a life-threatening clinical syndrome and a morphological manifestation of severe glomerular injury that is marked by a proliferative histological pattern ('crescents') with accumulation of T cells and macrophages and proliferation of intrinsic glomerular cells. Bollée and co-researchers show de novo induction of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in intrinsic glomerular epithelial cells (podocytes) from both mice and humans with RPGN. HB-EGF induction increases phosphorylation of the epidermal growth factor receptor (EGFR, also known as ErbB1) in mice with RPGN. In HB-EGF-deficient mice, EGFR activation in glomeruli is absent and the course of RPGN is improved. Autocrine HB-EGF induces a phenotypic switch in podocytes in vitro. Conditional deletion of the Egfr gene from podocytes of mice alleviates the severity of RPGN. Likewise, pharmacological blockade of EGFR also improves the course of RPGN, even when started 4 days after the induction of experimental RPGN. This suggests that targeting the HB-EGF-EGFR pathway could also be beneficial in treatment of human RPGN.

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Eitan Israeli

Capsule

Polymeric IgA1 controls erythroblast proliferation and accelerates erythropoiesis recovery in anemia

Anemia due to insufficient production of and/or response to erythropoietin (Epo) is a major complication of chronic kidney disease and cancer. The mechanisms modulating the sensitivity of erythroblasts to Epo remain poorly understood. Coulon et al. show that, when cultured with Epo at suboptimal concentrations, the growth and clonogenic potential of erythroblasts was rescued by transferrin receptor 1 (TfR1)-bound polymeric IgA1 (pIgA1). Under homeostatic conditions, erythroblast numbers were increased in mice expressing human IgA1 compared to control mice. Hypoxic stress of these mice led to increased amounts of pIgA1 and erythroblast expansion. Expression of human IgA1 or treatment of wild-type mice with the TfR1 ligands pIgA1 or iron-loaded transferrin (Fe-Tf) accelerated recovery from acute anemia. TfR1 engagement by either pIgA1 or Fe-Tf increased cell sensitivity to Epo by inducing activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling pathways. These cellular responses were mediated through the TfR1-internalization motif, YXXΦ. These results show that pIgA1 and TfR1 are positive regulators of erythropoiesis in both physiological and pathological situations. Targeting this pathway may provide alternate approaches to the treatment of ineffective erythropoiesis and anemia.

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Eitan Israeli

"A person without a sense of humor is like a wagon without springs – jolted by every pebble in the road"

Henry Ward Beecher (1813-1887), American preacher, social reformer and writer

"Besides the noble art of getting things done, there is the noble art of leaving things undone. The wisdom of life consists in the elimination of non-essentials"

Lin Yutang (1895-1976), Chinese writer, whose informal but polished style in both Chinese and English made him one of the most influential writers of his generation and his translations of classic Chinese texts into English were bestsellers in the West

Posterior Reversible Encephalopathy Syndrome Complicating Septic Shock

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KEY WORDS: posterior reversible encephalopathy syndrome (PRES), sepsis, infection, Crohn's disease

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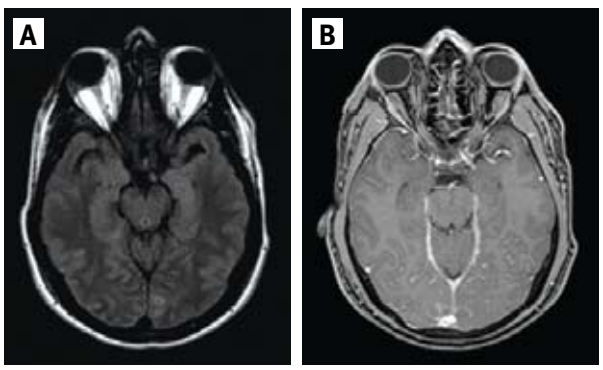
Posterior reversible encephalopathy syndrome is an acute neurologic injury characterized by unique computed tomography and magnetic resonance imaging findings. Clinical symptoms may range from headache, vision changes, paresis and altered mental status to generalized seizures and coma. In approximately 70% to 80% of patients moderate to severe hypertension is observed. PRES has been described in association with preeclampsia, autoimmune diseases, cancer chemotherapy and transplantation. Lately it was also described in the context of infections and severe sepsis [1,2]. PRES is usually characterized by reversibility of the clinical and radiological abnormalities once the underlying disease process is resolved. Since there is no specific therapy, stabilization of the hemodynamic status, eradication of the infectious agent, and control of seizures are the mainstay of its management [3,4].

PATIENT DESCRIPTION

A 33 year old man was admitted to the medical intensive care unit due to septic shock. Two days before admission he developed fever with chills but denied headache or any other localizing symptom. His past medical history was remarkable for stable Crohn's disease for which he underwent surgery at the age of 14 (ileal resection with ileo-colic anastomosis). He was not taking any medications. The patient was admitted following an episode of generalized seizure. On arrival his temperature was 38.6°C, blood pressure 80/26, heart rate 123, and saturation 90% while breathing room air. He was alert and oriented and his physical examination, chest X-ray and laboratory findings were uninformative. Intravenous metronidazole and ceftriaxone were initiated and he was admitted to the medical intensive care unit with a presumptive diagnosis of septic shock. Shortly after admission he developed a generalized seizure with left upward eye gaze deviation that lasted several minutes. Upon regaining consciousness he complained of complete loss of vision. Ocular reflexive response to motion was preserved but he denied any light perception. In addition, he demonstrated partial retrograde amnesia, confusion, and temporal disorientation with no motor or sensory loss. The course was complicated by multi-organ failure comprising hemodynamic instability requiring vasopressors, acute renal failure, disturbed liver functions and coagulopathy.

The abrupt onset of neurological symptoms preceded by a febrile illness had led to an initial working diagnosis of infectious viral encephalitis versus PRES. Since the latter is a diagnosis of exclusion, alternative considerations included central nervous system vasculitis, acute and subacute neurological disease (acute disseminated encephalomyelitis, cerebral venous thrombosis), cerebral hypoperfusion/ischemia, and mitochondrial disease such as the MELAS syndrome (**m**itochondrial **e**ncephalomyopathy, **l**actic **a**cidosis and **s**troke-like episodes). A lumbar puncture revealed a normal opening pressure. CT-angiography/venography of the head as well as the cerebrospinal fluid analysis were within normal limits. Brain MRI showed bilateral occipital lobes high signal on T2 and flair [Figure A], and abnormal enhancement after contrast administration on T1 [Figure B]. Chest and abdomen CT without contrast were significant for bilateral pleural effusions and well-healed ileal resection and ileo-colic anastomosis. All

PRES = posterior reversible encephalopathy syndrome



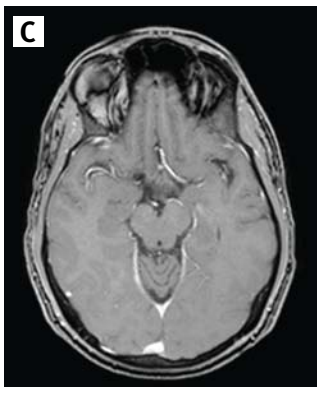
[A] Axial flair study (on day 2) demonstrating high signal intensity of the occipital lobes bilaterally
[B] T1 after contrast administration demonstrating minimal enhancement of the abnormal area

cultures were sterile (blood, urine, sputum, CSF). Serologic evaluation for *Ricketsia typhi* and *conorii*, Q fever and Brucella were negative, as were human immunodeficiency virus and Epstein-Barr virus; and tests for immunoglobulin M, antinuclear antibodies, C-reactive protein, rheumatoid factor, and cytoplasmic and perinuclear antineutrophil cytoplasmic antibodies were normal. Electroencephalogram showed pathological bilateral occipital slowing without alpha wave response upon eye opening. Normal lumbar puncture, negative serologies, sterile blood cultures and MRI images consistent with bilateral occipital region edema excluded most of the differential diagnoses, leading by exclusion to the diagnosis of PRES.

With supportive care and antibiotic therapy the multi-organ failure gradually resolved, followed by significant neurological improvement and return of near-normal sight on ophthalmologic examination. A follow-up MRI prior to discharge (3 weeks after his initial admission) revealed a significant decrease in the regions of high signal on T2 and flair. T1 post-contrast administration showed diminished enhancement due to reconstitution of the blood-brain barrier [Figure C]. Today, 3 years after his initial presentation, the patient is well with normal vision.

CSF = cerebrospinal fluid

[C] Follow-up T1 image after contrast administration study reveals decreased enhancement without any signs of delayed ischemic sequel



COMMENT

The case under discussion illustrates the following important clinical issues: a) the clinical spectrum and radiological characteristics of PRES; b) its association with sepsis; and c) the tip of the iceberg phenomenon, i.e., under-diagnosis, since the true incidence of PRES is unknown among critically ill sedated patient.

- ***Clinical spectrum and radiological characteristics of PRES.*** The first to describe a reversible neurological syndrome characterized by headache, confusion, seizures, and impaired vision in associated with typical neuroimaging changes were Hinchey et al. in 1996 [1]. They coined the term posterior reversible leukoencephalopathy. Since this term is rather misleading as both the white and grey matter can be affected, the current acronym of PRES was adopted. The diagnosis of PRES is based on clinical history, characteristic imaging, and proof of reversible viability. Typically, PRES is an acute encephalopathy manifesting as vomiting, headache, altered mental status, seizures and visual impairment. The full neurological presentation is often heralded by convulsions, the most common presenting symptom in up to 74% of patients [3]. Convulsions are usually generalized and multiple. Status epilepticus is not uncommon. Visual disturbances are one of the hallmarks of this entity; they are found in up to 20% of patients [3] and may range from hemianopsia to cortical blindness as described in the present case [4]. Recognition of the characteristic imaging findings is the key diagnostic clue. Computed tomography can be used to detect hypodense lesions typical of posterior encephalopathy, but magnetic resonance imaging is the gold standard. In the acute phase, MRI reveals hyperintense signals on both T2-weighted and flair sequences and either iso- or hypointense on T1-weighted images, including both gray and white matter. The characteristic pattern associated with PRES resembles the brain watershed zones, with the cortex and subcortical white matter involved to varying degrees. The parietal and occipital lobes are predominantly affected, similar to the case under discussion, followed by the frontal lobes, the inferior temporo-occipital junction and the cerebellum [5]. The findings in the present case may initially be attributed to cerebral hypoperfusion and ischemia. However, in the diffusion weighted MRI, a distinctive feature of ischemic lesions was lacking. Moreover, the quick and complete resolution of the clinical and radiological findings in a young man with no atherosclerotic disease provides the final evidence for the probable nature of the process. Taken together, the clinical findings and the course are highly suggestive of PRES. Treatment of patients with PRES must focus on prompt correction of the mean arterial blood pressure, proper hydration, adequate oxygenation, and correction of electrolyte disturbances. Removal of precipitating factors is of utmost importance in order to halt the hemodynamic and inflammatory cascade that perpetuates the pathophysiologic process of PRES. Treatment of seizures and especially status epilepticus is crucial. When available, the use of continuous electroencephalographic monitoring is advised. Prognosis is usually favorable, and with prompt diagnosis and adequate therapy most patients are expected to fully recover within a few weeks [4].
- ***Pathophysiology and association with infection and sepsis.*** PRES is almost always seen in the setting of significant systemic processes. The most common etiologies are acute severe hypertension, toxemia of pregnancy, and immunosuppressive therapy (with cyclosporine as the most often reported drug). Other less frequent etiologies include autoimmune dis-

ease, cancer chemotherapy and, as reported recently, infection and sepsis. In a review of 120 patients with PRES, hypertension was the most common etiology and was found in 61%, followed by immunosuppressive therapy (19%). Although a well and commonly described etiology, toxemia of pregnancy was the cause in only 6% of the patients reviewed, while sepsis was the cause in 7%. Forty-five percent of the patients described had a history of autoimmune disease, most commonly thrombotic thrombocytopenia purpura and systemic lupus erythematosus. Interestingly, 6% had Crohn's disease similar to our patient [3].

The pathophysiology of PRES is still poorly understood. The initial cytotoxic theory suggested that vasoconstriction and hypoperfusion lead to brain ischemia and subsequent vasogenic edema. The vasogenic theory proposed that severe hypertension leads to failed autoregulation, subsequent hyperperfusion with endothelial dysfunction, and vasogenic edema. This concept is corroborated by the fact that significant hypertension is found among 50% of patients. Nonetheless, PRES is seen in increasing frequency without hypertension, as in our patient. Moreover, even when hypertension is documented, the mean arterial blood pressure does not typically reach the limit of failed autoregulation. Indeed, in a retrospective review of 25 patients diagnosed with PRES in the setting of severe infection, 40% had normal blood pressure [2]. The present case illustrates the association between severe infection, occurring in a patient with autoimmune disease, and PRES. It further suggests that it may complicate the course of hypotensive patients with sepsis. Current research has led to a new unifying theory explaining the pathogenesis of PRES [5]. In the majority of patients with PRES an underlying systemic cytokine-mediated inflammatory response is present. T cell activation and elevated inflammatory cytokines (tumor necrosis factor-alpha, interleukin 1, interferon-gamma and interleukin 6) are common. Cytokines upregulate endothelial surface antigens (P-selectin, E-selectin, intercellular and vascular cellular adhesion molecule-1) and increased leukocyte adherence leading to microcirculatory dysfunction. The developing vasculopathy is accompanied by altered intrinsic vascular tone and vasodilatation. The common final pathway culminates in brain and systemic hypoperfusion and the development of vasogenic edema.

- ***The tip of the iceberg:*** Recognizing neurological impairment in the critically ill may be difficult. Visual disturbances, which are distinctly characteristic, are even more elusive in the sedated patient. PRES may be more frequent than previously reported. It has a broad clinical spectrum, and differentiating PRES from other more common causes of neurological impairments may be difficult since PRES may have normal findings on initial CT, highlighting the importance of MRI when the clinical context is suggestive. Thus, PRES should be included in the differential diagnosis of unexplained seizures and altered mental status in the critically ill patient. As with other rare clinical entities, a high index of suspicion may lead to earlier diagnosis and appropriate treatment.

In summary, this case serves as an important reminder that PRES may complicate the course of sepsis. The exact incidence of PRES is difficult to determine among the critically ill, but conceivably a certain proportion of patients with critical illness-associated cognitive dysfunction may actually represent a form of PRES. Even though there is no specific therapy, early diagnosis is probably important and requires a high index of suspicion.

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"We're here to put a dent in the universe"

Steve Jobs (1955-2011), American entrepreneur and inventor, co-founder, chairman, and chief executive officer of Apple Inc

"A cult is a religion with no political power"

Tom Wolfe (born 1931), American author and journalist

"History is a vast early warning system"

Norman Cousins (1915-1990), American political journalist, author, professor, and world peace activist

Rare Presentations of Congenital Hypothyroidism

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KEY WORDS: congenital hypothyroidism, neonatal screening, abdominal distension, weak pulse, newborn infant

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Congenital hypothyroidism is mostly asymptomatic at birth. It is usually detected by targeted neonatal screening for CH that is performed after birth. Nonetheless, there are reports of rare and vague presentations of CH. These include protracted icterus, large tongue, abdominal distension, skin mottling, muscle hypotonia, prolonged gestation, large posterior fontanel, respiratory distress, hypothermia, peripheral cyanosis, hypoactivity, poor feeding, lag in onset of stooling, abdominal distension with vomiting, edema [1,2] and chylothorax [3]. We describe here two newborn infants with rare clinical presentations that could be attributed to CH. The crucial role of neonatal screening in the diagnosis of CH is highlighted.

PATIENT DESCRIPTIONS

PATIENT 1

A 2550 g term female infant, born to a group B Streptococcus-carrier mother, had abdominal distension at discharge examination [Figure A]. The infant nursed well without vomiting and passed meconium. There was no organomegaly or palpable abdominal masses. Sepsis workup was performed and empiric ampicillin and cefotaxime were started.

CH = congenital hypothyroidism

A decompressive nasogastric tube was inserted and intravenous fluids were initiated. Abdominal radiograph was normal except for mild bowel distension [Figure B]. Sepsis workup was negative. Abdominal ultrasonography was normal. On day 4 of life, the Israeli National Neonatal Screening Program reported a high thyroid-stimulating hormone level (> 400 mU/L). Venous sample of free thryoxine 2.4 pmol/L (normal 12–22) and TSH > 100 mU/L (normal < 10 mU/L) were compatible with CH. Thyroid hormone supplementation was started and 4 days later abdominal distension subsided; the infant nursed well and was discharged home. Follow-up 2 weeks later revealed a healthy infant with normal FT4 and TSH.

PATIENT 2

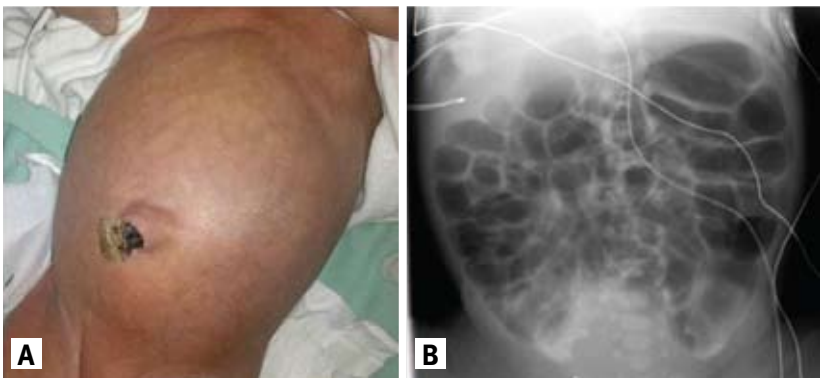
A term female infant weighing 4110 g presented with a systolic murmur and barely palpable pulses. Blood pressure (four limbs) was normal. Echocardiography was normal except for patent foramen ovale. The infant was asymptomatic but pulses remained weak. At day 4, the Israeli National Neonatal Screening Program reported a high TSH level (> 400 mU/L). The venous sample of FT4 = 2 pmol/L and TSH > 100 mU/L was compatible with CH. Thyroid hormone supplementation was started and 10 days later the pulses became normal and were easily palpable.

TSH = thyroid-stimulating hormone
FT4 = free thyroxine

COMMENT

In this report we wish to raise the awareness of neonatologists regarding uncommon clinical presentations of CH, where the clinical signs disappeared following thyroid hormone supplementation. In the first patient, abdominal distension was likely the sole sign of CH and was apparently caused by decreased intestinal motility often observed with CH [2]. In the second patient, weak pulses could have been due to CH which has been associated with decreased beat-to-beat variability of fetal heart rate [4] and with decreased left ventricular function [5].

Abdominal radiographs: **[A]** Significant bowel distension at discharge examination (day 2), **[B]** Mild bowel distension (day 4).



These cases also demonstrate the crucial role of neonatal screening, which allowed early and timely treatment of CH.

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Capsule

Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation

Tissue factor, the initiator of the coagulation cascade, mediates coagulation factor VIIa-dependent activation of protease-activated receptor 2 (PAR2). Badeanlou et al. delineate a role for this signaling pathway in obesity and its complications. Mice lacking PAR2 (F2rl1) or the cytoplasmic domain of tissue factor were protected from weight gain and insulin resistance induced by a high-fat diet. In hematopoietic cells, genetic ablation of tissue factor-PAR2 signaling reduced adipose tissue macrophage inflammation, and specific pharmacological inhibition of macrophage tissue factor signaling rapidly ameliorated insulin resistance. In contrast, non-hematopoietic cell tissue factor-VIIa-PAR2 signaling specifically promoted obesity. Mechanistically, adipocyte tissue factor cytoplasmic domain-dependent VIIa signaling suppressed Akt phosphorylation with concordant adverse transcriptional changes of key regulators of obesity and metabolism. Pharmacological blockade of adipocyte tissue factor in vivo reversed these effects of tissue factor-VIIa signaling and rapidly increased energy expenditure. Thus, inhibition of tissue factor signaling is a potential therapeutic avenue for improving impaired metabolism and insulin resistance in obesity.

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Eitan Israeli

Capsule

Melanopsin signaling in mammalian iris and retina

Non-mammalian vertebrates have an intrinsically photosensitive iris and thus a local pupillary light reflex (PLR). In contrast, it is thought that the PLR in mammals generally requires neuronal circuitry connecting the eye and the brain. Xue et al. report that an intrinsic component of the PLR is in fact widespread in nocturnal and crepuscular mammals. In mouse, this intrinsic PLR requires the visual pigment melanopsin; it also requires PLCβ4, a vertebrate homologue of the Drosophila NorpA phospholipase C which mediates rhabdomeric phototransduction. The Plcb4–/– genotype, in addition to removing the intrinsic PLR, also essentially eliminates the intrinsic light response of the M1 subtype of melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (M1-ipRGCs), which are by far the most photosensitive ipRGC subtype and also have the largest response to light. Ablating in mouse the expression of both TRPC6 and TRPC7, members of the TRP channel superfamily, also essentially eliminated the M1-ipRGC light response but the intrinsic PLR was not affected. Thus, melanopsin signaling exists in both iris and retina, involving a PLCβ4-mediated pathway that nonetheless diverges in the two locations.

Nature 2011; 479: 67

Eitan Israeli

"Every man is a damned fool for at least five minutes every day. Wisdom consists in not exceeding the limit"

Elbert Hubbard (1856-1915), American writer, publisher, artist and philosopher

"Lots of people want to ride with you in the limo, but what you want is someone who will take the bus with you when the limo breaks down"

Oprah Winfrey (born 1954), American talk show host and philanthropist