Antibodies to Various Mycoplasmas in Patients with Coronary Heart Disease

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ABSTRACT: **Background:** Clinical and epidemiologic features of coronary heart disease may not be explained solely by established risk factors. The role of infectious pathogens in the development and rupture of atherosclerotic plaques remains elusive but an association between *Chlamydia pneumoniae, Mycoplasma pneumoniae* and CHD has been reported previously.

Objectives: To determine whether there is an association between mycoplasmal infections and CHD.

Methods: We conducted a prospective cohort analysis of 150 consecutive hospitalized patients with CHD (85 with acute coronary syndrome and 65 admitted for unrelated reasons) and 98 healthy blood donors. Antibody titers for *Mycoplasma pneumoniae, M. fermentans, M. hominis* and *Ureaplasma urealyticum* were measured with the agglutination test or specific enzyme-linked immunosorbent assay in all three groups of patients.

Results: Analysis of the antibody titers did not reveal any significant difference in the presence of mycoplasmal antibodies between the patients with ACS, patients with known stable CHD hospitalized for non-CHD reasons, and healthy blood donors.

Conclusions: Determination of specific antibodies did not reveal a significant association among different types of mycoplasmal infection and CHD.

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KEY WORDS: *Mycoplasma pneumoniae, Mycoplasma fermentans, Mycoplasma hominis, Ureaplasma urealyticum,* acute coronary syndrome, coronary heart disease, atherosclerosis

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Factors other than the conventional risk factors may contribute to the development of atherosclerosis [1-3]. Since inflammation plays an important role in the pathogenesis of atherosclerosis [1,3,4], the potential role of infectious agents in the initiation or modulation of atherosclerosis has been investigated. Several seroepidemiologic and immunohistochemical studies have demonstrated an association between microbial infections, particularly *Chlamydia pneumoniae,* and atherosclerosis [1,5]. Mycoplasmas, the smallest self-replicating wall-less bacteria, are parasites in humans, animals and plants. They are ubiquitous and tend to establish chronic infections which are accompanied by alterations of immune reactivity [6]. The involvement of *Mycoplasma pneumoniae* in cardiovascular diseases has been previously reported, and several investigators have pointed to a possible association, along with *Chlamydia pneumoniae*, as risk factors for embolization and myocardial infarction [7]. However, the results are controversial [8-11]. There have been no reports regarding other human mycoplasmas. In the present study a possible relationship between mycoplasmal infections and CHD was evaluated by measuring the level of specific antibodies to several human mycoplasmas in CHD patients.

CHD = coronary heart disease
ACS = acute coronary syndrome

PATIENTS AND METHODS

A prospective cohort study of 150 consecutive patients with coronary heart disease hospitalized in Soroka University Medical Center, and 98 healthy blood donors was performed. Of the 150 patients with CHD, 85 were admitted with an ACS and 65 for reasons unrelated to CHD. The discharge diagnoses (ICD-9) for identifying subjects with coronary heart disease was used. Patients with active atherosclerotic cerebral and peripheral arterial disease (admitted due to stroke or peripheral vascular disease) were excluded. The study was approved by the Institutional Review Board.

MEASUREMENT OF ANTI-MYCOPLASMAL ANTIBODIES

Anti-*Mycoplasma pneumoniae* antibodies in patients' sera were measured by the microagglutination test (Fujirabo Kit, Japan). Titers of 1:80 and above were considered positive (Fujirabo Kit). Anti-*Ureaplasma urealyticum* and anti-*Ureaplasma parvum* antibodies were measured by a specific enzyme-linked immunosorbent assay routinely used in our National Center for Mycoplasma (Clalit Health Services). A titer above 1:160 was considered positive. Anti-*Mycoplasma fermentans* and anti-*Mycoplasma hominis* antibodies were measured with a highly specific ELISA used in our center.

ELISA = enzyme-linked immunosorbent assay

Table 1. Comparison of clinical characteristics of patients with ACS and stable CAD

Characteristics Mean (SD)/ n (%)	Acute coronary syndrome (n=85)	Stable coronary heart disease (n=65)	*P* value
Age (yrs)	62.1 (8.1)	61.3 (7.2)	0.5
Gender (male)	68 (80)	47 (72.3)	0.4
Diabetes mellitus	33 (38.8)	26 (40)	1
Chronic renal failure	10 (11.8)	8 (12.3)	1
MI in the past	17 (20)	18 (27.7)	0.3
History of CABG	13 (15.3)	21 (32.3)	0.023
History of PCI	9 (10.6)	17 (26.2)	0.02
Heart failure	17 (20)	9 (13.8)	0.4
Atrial fibrillation	5 (5.9)	4 (6.2)	1
Significant valvular disease	4 (4.7)	9 (13.8)	0.09
ICD	1 (1.2)	2 (3.1)	0.6
History of stroke	4 (4.7)	4 (6.2)	0.7
Hypertension	47 (55.3)	38 (58.5)	0.8
Rheumatologic disease	1 (1.2)	3 (4.6)	0.3
Malignancy	5 (5.9)	5 (7.7)	0.7
COPD	3 (3.5)	6 (9.2)	0.18
Pneumonia	2 (2.4)	1 (1.5)	1
Other lung disease	4 (4.7)	2 (3.1)	0.7
Recurrent urinary tract infection	1 (1.2)	2 (3.1)	0.6

Numbers with a *P* value of < 0.05 were considered significant

CABG = coronary artery bypass graft, COPD = chronic obstructive pulmonary disease ICD = implantable cardioverter defibrillator, MI = myocardial infarction, PCI = percutaneous coronary intervention

The cutoff values (for each mycoplasma) were previously determined by comparing infected patients with the healthy population [12,13]. The cutoff values for *M. fermentans* and for *M. hominis* were 0.687 and 0.876 optical density, respectively. These values were calculated as the mean plus 2 SD (standard deviations) in the healthy population.

STATISTICAL ANALYSIS

The results are presented as the mean (SD) for continuous variables and as the total number of patients (percentage of total patients) for categorical data. The *t*-test was used for comparison of the continuous variables or chi-square test for categorical data with the use of Fisher's exact test if needed. A two-sided *P* value < 0 .05 was considered statistically significant.

RESULTS

Characteristics of the patients with coronary disease are shown in Table 1. As shown in Table 2, there were no significant differences in the prevalence of anti-mycoplasmal antibodies (four different species of human mycoplasmas) between the patients with coronary heart disease and the group of healthy donors. Also, as shown in Table 3, there was no difference in positive antibody response between patients hospitalized due to ACS and patients with stable CHD.

Table 2. Prevalence of anti-mycoplasma antibodies in patients with coronary heart disease and healthy donors

Antibodies	Coronary heart disease (n=150)	Healthy donors (n=98)	*P* value
Ureaplasma urealyticum	26/145 (17.9%)	13/98 (13.3%)	0.43
Mycoplasma pneumonia	20/150 (13.3%)	21/98 (21.2%)	0.14
Mycoplasma fermentans	5/150 (3.3%)	3/98 (3.1%)	1.0
Mycoplasma hominis	9/150 (6.0%)	10/97 (10.3%)	0.32

Table 3. Prevalence of anti-mycoplasma antibodies in patients with acute coronary syndrome and stable coronary heart disease

Antibodies	Acute coronary syndrome (n=85)	Stable coronary heart disease (n=65)	*P* value
Ureaplasma urealyticum	15/81 (18.5%)	11/63 (17.5%)	1.0
Mycoplasma pneumonia	12/85 (14.3%)	8/65 (12.3%)	0.91
Mycoplasma fermentans	2/85 (2.4%)	3/65 (4.6%)	0.65
Mycoplasma hominis	5/85 (6.0%)	4/65 (6.2%)	1.0

DISCUSSION

It has been previously demonstrated that inflammation plays an important role in the pathogenesis of atherosclerosis [1,3,4] and activated macrophages and T lymphocytes are present in atherosclerotic plaques, resulting in an immune-mediated process [1].

Several seroepidemiologic and immunohistochemical studies have demonstrated an association between microbial infections and atherosclerosis [1,5,14]. In addition, infectious agents were detected in atheromas by molecular techniques (mainly polymerase chain reaction) [14,15]. A specific T cell response, both in atheromatous plaque and in peripheral blood, was also detected [1,7].

Several microorganisms have been associated with atherosclerotic diseases [5,14,15], but the results have been controversial and it is not clear whether infectious agents play an active role in atherogenesis or whether they are passive inhabitants of the plaque [6,16,17]. Previous reports have noted the involvement of *Mycoplasma pneumoniae* in cardiovascular diseases [8-11,18], although cardiac complications

associated with *M. pneumoniae* are relatively uncommon (1%–8.5% in persons with serologic evidence of infection) [19]. Pericarditis, myocarditis and pericardial effusion with and without cardiac tamponade have all been described [18], and the organism has been detected by PCR in pericardial fluid as well as in ruptured atherosclerotic plaques and stenotic heart valves [8,11,19]. According to one study, almost half the patients with *M. pneumoniae* infection had symptoms or signs of heart abnormalities at an average of 16 months later [19]. There were no reports on other human mycoplasmas.

Mycoplasma species are possible candidates to be involved in diseases due to their ability to induce chronic disease states. Chronicity develops due to several reasons: First, they adhere tightly to the host cell membrane, or alternatively become intracellular residents, and clearing of the organism is therefore extremely difficult. Second, mycoplasmas are notorious for their fast and elaborated variation of their surface lipoproteins, and therefore evade the immune system [18]. Third, these bacteria exert immunomodulatory effects, namely, altering the functions of several cells of the immune machinery, e.g., activation of macrophages and T lymphocytes [6,20]. These cells are known to play an important role in the atheromatous plaque formation [21,22]. Also, some mycoplasmas (*Ureaplasma urealyticum*) also possess phospholipase A [23], an enzyme that plays a pivotal role in the transformation of macrophages into foam cells, a major component of the atheromatous plaque [24]. In addition, some mycoplasmas cause accumulation of H_2O_2 and other oxygenated radicals that may increase the oxidation in the plaque. Mycoplasmas induce cytokine production by macrophages, especially tumor necrosis factor-alpha, interleukin-6 and IL-1β [6] – cytokines that are involved in atheromatous plaque formation [21, 22]. All the above may suggest that mycoplasmal infection might act in a pro-atherogenic fashion. In one study *M. pneumoniae* was found in almost all coronary atheromas and it was suggested that large amounts of *M. pneumoniae* and *Chlamydia pneumoniae* may be necessary for the development of plaque instability [11].

In the present study we measured antibodies in patients' sera. There were no significant differences in the prevalence of positive antibody response to any mycoplasma among patients with CHD and healthy subjects or between patients with acute coronary syndrome and stable coronary heart disease. These results do not exclude the possibility that mycoplasmas are involved in the development of atheromas. It merely suggests that determining the level of antibody to mycoplasma, usually used to identify mycoplasmal infection, is not the appropriate method to evaluate an association between the infectious agent and CHD. This is probably due to the intracellular localization of mycoplasmas in the macrophages, which render them less exposed to the immune humoral response, namely production of specific antibodies [25].

PCR = polymerase chain reaction
IL = interleukin

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Capsule

Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT$_{2C}$ receptors

Muscle paralysis after spinal cord injury is partly caused by a loss of brainstem-derived serotonin (5-HT), which normally maintains motoneuron excitability by regulating crucial persistent calcium currents. Murray et al. examined how over time motoneurons compensate for lost 5-HT to regain excitability. The authors found that, months after a spinal transection in rats, changes in post-transcriptional editing of 5-HT$_{2C}$ receptor mRNA led to increased expression of 5-HT$_{2C}$ receptor isoforms that are spontaneously active (constitutively active) without 5-HT. Such constitutive receptor activity restores large persistent calcium currents in motoneurons in the absence of 5-HT. They show that this helps motoneurons recover their ability to produce sustained muscle contractions and ultimately enables recovery of motor functions such as locomotion. However, without regulation from the brain, these sustained contractions can also cause debilitating muscle spasms. Accordingly, blocking constitutively active 5-HT$_{2C}$ receptors with SB206553 or cyproheptadine, in both rats and humans, largely eliminates these calcium currents and muscle spasms, providing a new rationale for antispastic drug therapy.

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Eitan Israeli

Capsule

Biology and pathogenesis of chikungunya virus

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus responsible for a recent, unexpectedly severe epidemic in countries of the Indian Ocean region. This outbreak was also implicated with a significant rise in occurrence of Guillain-Barré syndrome in Reunion Island. Although many alphaviruses have been well studied, little was known about the biology and pathogenesis of CHIKV at the time of the 2005 outbreak. Over the past 5 years a multidisciplinary effort has aimed at deciphering the clinical, physiopathologic, immunologic and virologic features of CHIKV infection. Schwartz & Albert review some of the most recent advances in our understanding of the biology of CHIKV and its interactions with the host.

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Eitan Israeli

Capsule

Interrelations among gut flora inhabitants

The influence of intestinal flora on the physiology of the entire host organism has only recently begun to be appreciated. The mammalian gut is not just home to billions of bacteria and archaea, but often harbors much larger creatures such as protozoa, nematodes, and tapeworms. Anticipating a web of interactions among these organisms, Hayes and collaborators investigated the relationship between a common nematode parasite of mice, *Trichuris muris*, and its dependency on the occurrence of enterobacteria, such as *Escherichia coli* and *Salmonella typhimurium.* The worms were found to be dependent on the presence of bacteria for hatching, and hatching was triggered by the presence of bacterial surface structures called fimbriae, which bind to proteins at the poles of the worms' eggs.

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Eitan Israeli

A Prospective Follow-Up of Two 21/7 Cycles Followed by Two Extended Regimen 84/7 Cycles with Contraceptive Pills Containing Ethinyl Estradiol and Drospirenone

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ABSTRACT: **Background:** Continuous use of combined oral contraceptives is currently attracting growing interest as a means of improving menstrual related symptoms and reducing the number of bleeding days.

Objectives: To evaluate bleeding patterns, menstrual symptoms and quality of life with an extended 84/7 oral contraceptive regimen versus 21/7 cycles.

Methods: In two consecutive run-in cycles, 30 µg ethinyl estradiol and 3 mg drospirenone tablets taken on days 1–21 were followed by a tablet-free period from days 22 to 28 of each cycle and then by two 84 day cycles of pill use with a 7 day tablet-free interval. The primary outcome was the total number of bleeding/spotting days. Secondary outcomes were severity of daily symptoms, general well-being determined by the PGWBI questionnaire, and overall treatment satisfaction.

Results: Of the 137 women invited to participate in the study 109 (aged 18–40 years) were enrolled. The number of bleeding days decreased by about one-third from a calculated 31.8 days of bleeding under a cyclic 21/7 regimen to an expected total of 21.8 days for the extended 84/7 regimen. The incidence of menorrhagia, intermenstrual bleeding, dysmenorrhea, abdominal bloating, breast tenderness, depressive moods and irritability – when compared at enrollment and at the end of the second extended study period – was significantly lower ($P < 0.005$) among women on the continuous pill regimen. The median (range) global PGWBI scores were not substantially different before and after the extended use cycles: 78.2 (39.1–96.4) and 77.3 (30.9–96.4), respectively. Body weight and skin condition also remained constant. At the completion of the study: 65.5% of the women were either highly satisfied (41.4%) or satisfied (24.1%) with the extended regimen.

Conclusions: The extended 84/7 regimen was found to be satisfactory for the majority of participants and was associated with a decrease in the number of bleeding days and an improvement in menstrual symptoms compared to 21/7 cycles.

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KEY WORDS: combined hormonal contraceptives, non-bleeding contraceptives, menorrhagia, continuous administration, spotting, menstrual symptoms

Extended use of combined oral contraceptives, omitting the hormone-free interval, has a favorable effect for many women by reducing the frequency of troublesome withdrawal bleeding [1]. It was shown to be associated with only slight breakthrough bleeding and relatively weak withdrawal bleeding episodes [1]. Continuous administration of combined oral contraceptives is, therefore, often recommended to women to avoid menstrual symptoms such as migraine headache [2], endometriosis-associated pelvic pain [3,4], premenstrual symptoms [5] and the inconvenience associated with menses [5,6]. Avoidance of these unpleasant symptoms may be a more powerful motivator for women to comply with oral contraceptives than the many known major health benefits, such as reduction in the risk of ovarian cancer. Few studies, however, have compared the safety, efficacy, side effects and potential advantages and disadvantages of continuous versus traditional administration of oral contraceptives [6-8]. Furthermore, an observational non-controlled study has suggested that women who took, continuously, a contraceptive pill containing 30 µg ethinyl estradiol combined with 3 mg drospirenone continuously for between 42 and 126 days seemed to have benefited from this progestin's anti-mineral corticoid activity (e.g., reduced breast tenderness, edema, bloating) and anti-androgenic activity (e.g., positive effects on the skin) [9]. Additional reports have shown that the continuous use of a 30 µg ethinyl estradiol and 3 mg drospirenone formulation over 126 days was safe, efficacious, well accepted by the users and resulted in a considerable reduction in bleeding [10,11].

A recent systematic Cochrane review of randomized controlled trials found that the contraceptive efficacy and compliance were similar for the continuous-use group versus the cyclic-use group [12]. The rate of discontinuation for any reason and specifically for bleeding problems was low in each group, and the participants reported high levels of satisfaction with both dosing regimens. Most studies found that bleeding patterns were either equivalent or improved with continuous-dosing regimens. For example, the continuous-dosing group in the study by Edelman et al. [12] had a greater reduction of menstrual-associated symptoms, including headaches, genital irritation, tiredness, bloating, and menstrual pain.

The authors of the Cochrane review commented that future studies on continuous or extended cycle use of combined oral contraceptives for contraception should choose a previously proven type of pill and dosing regimen while paying more attention to participant satisfaction and menstruation-associated symptoms. The aim of the present study was therefore to examine in a prospective manner the hypothesis that women taking combined oral contraceptives containing drospirenone, without a hormone-free interval for up to 84 days, have a favorable rate of side effects and bleeding days leading to improved quality of life and high satisfaction rates.

SUBJECTS AND METHODS

The purpose of this study was to evaluate bleeding patterns and acceptability of an extended 84/7 oral contraceptive regimen using a combined pill containing 30 μg ethinyl estradiol and 3 mg drospirenone taken for 84 continuous days during two consecutive cycles compared to two preceding 21/7 cycles using the same pills.

Nine outpatient gynecology clinics participated. Women between the age of 18 and 40 who requested oral contraceptive therapy, were willing and able to give informed consent, and had no medical contraindication to combined oral contraceptive therapy were invited to participate.

Each subject received, in two consecutive run-in cycles, an oral dose of 30 μg ethinyl estradiol and 3 mg drospirenone (Yasmin, Bayer Schering AG, Berlin, Germany) on days 1–21, followed by a tablet-free period from day 22 to 28 of each cycle. This was immediately followed by two extended cycles of 84 continuous days of pill administration with a 7 day tablet-free interval. The enrolled subjects received free oral contraceptives for the duration of the study (an unrestricted gift from Bayer Schering AG). They were all requested to return to the clinic after completion of the run-in cycles and after finishing the first and second extended cycles. The Institution Review Board of the Sheba Medical Center approved the study protocol and all participants signed a written informed consent.

The primary outcome was the total number of bleeding/spotting days during the 6 month course of treatment. The mean number of reported bleeding days was analyzed for each of the two extended treatment cycles and for the overall duration of the study. Secondary outcomes included the severity of selected daily symptoms and overall treatment satisfaction. The participants were also asked to describe their skin condition at enrollment, and any reported changes that occurred during the study phases were recorded. The somatic symptoms of the participants in the current study were rated every day, and changes in the total score served as a parameter to test the efficacy of the regimen.

The study women's mood state was assessed using the Psychological General Well-Being Index [13] before and after the extended-use cycles. The PGWBI is a brief self-administered questionnaire with 20 items rated on a six-point scale, where a higher score indicates a better quality of life and measures six mood states (anxiety, depressed mood, positive well-being, self-control, general health, vitality). The six mood states are scored as follows: 25 for anxiety, 20 for positive well-being and vitality, and 15 for the others. The PGWBI was completed by the patients themselves.

At enrollment, the women were provided with daily diaries and asked to document any occurrence of vaginal bleeding and side effects. Vaginal bleeding was categorized as either spotting or bleeding. Spotting was defined as a light flow that did not necessitate sanitary protection, and bleeding was defined as a heavier flow that did require sanitary protection. Subjects reporting bleeding were asked to classify it each day as light, moderate or heavy.

Participants were also asked to note any symptoms of headache, nausea, bloating, breast tenderness, irritability, depressive moods and menstrual pain, as well as any pills that were missed. They completed a satisfaction questionnaire at the conclusion of each extended-regimen cycle. Overall satisfaction with bleeding patterns and symptom severity were recorded on a 100 mm visual analog scale.

Data collection began at the time of study entry and was completed during clinic visits for all patients within 12 months. Cycle regularity, symptoms related to menstrual regularity, and associated medical problems and complications were recorded. This information was entered into a patient logbook and later transferred onto a computerized form. Study discontinuation, conception, and adverse events were noted. In the event of withdrawal from the study, the woman's accumulated data were analyzed up to that point.

STATISTICAL ANALYSIS

Our sample size was based on expected differences in the primary outcome of total bleeding days during extended pill use

PGWBI = Psychological General Well-Being Index

in comparison to a hypothetical period of six 21 day pill cycles. The statistical analysis was done with the chi-square or Fisher exact tests for categorical variables and the unpaired Student's *t*-test for continuous factors. A correlation between PGWBI scores, before and after the extended regimen, was performed using a Spearman correlation test. All the tests were two-sided. A P value of < 0.05 was accepted as significant.

RESULTS

Of the 137 women invited to participate in the study, 109 women consented and were enrolled from September 2004 through February 2006. Their mean ± SD age was 27.5 ± 5.3 years; 32 (29.4%) were multiparas and 77 (70.6%) were nulliparas. Most of them were white-collar workers (64.2%) and students (29.4%) and a few were manual workers (2.7%) or unemployed (3.7%). Almost one-third were married (30.3%) for a mean ± SD of 5.4 ± 3.5 years. About one-fourth were smokers (26.6%) who smoked a mean of 6.5 ± 4.4 cigarettes daily; only two were considered heavy smokers (>15 cigarettes a day). Prior use of oral contraceptives was reported by 39 subjects (35.8%) for a mean of 6.1 ± 4.4 years.

Sixty-eight of the 109 enrolled women (62.4%) completed the entire study protocol. The most common reasons given for discontinuation were loss of interest (8.3%), bleeding (6.4%), weight gain (6.4%), dissatisfaction with the study (5.6%), fear of participating in research (4.6%), and wishing to conceive (2.8%).

The number of bleeding days decreased by about one-third compared to the two run-in cycles [Table 1]. The calculation was based on the assumption that there would be 28.6 (4.4 x 6.5) days of withdrawal bleeding and 3.2 (0.5 x 6.5) days of breakthrough bleeding, yielding a total of 31.8 days of bleeding under a cyclic 21/7 regimen lasting for 182 days (i.e., during 6.5 cycles). This was compared to an expected total of 21.8 days of bleeding under a continuous regimen based on an average of the two extended cycles (totaling 182 days) with a mean of 9.7 (4.9 ± 4.8) days of withdrawal bleeding and 12.1 (7.0 ± 5.1) days of breakthrough bleeding.

The incidence of menorrhagia, intermenstrual bleeding, dysmenorrhea, abdominal bloating, breast tenderness, depressive moods and irritability – at enrollment compared with the end of the second extended study period – was significantly lower ($P < 0.005$) among women under the continuous pill regimen [Table 2]. There was no significant change in body weight during the study period: the mean weight of the cohort had been 60.0 ± 10.1 kg at enrollment, 60.3 ± 10.2 kg after the two run-in 21/7 day cycles, 60.7 ± 12.7 kg after the first extended contraceptive cycle, and 59.3 ± 16.6 kg after the second extended contraceptive cycle. However, while 20 of the participants gained 2 kg or more from enrollment to the completion of the study, only 11 women lost 2 kg or more during the same period. The participants' skin condition did not change markedly during the study period, with 84.7% reporting no difference, 9.7% reporting improvement and 5.6% reporting worsening, at the end of the study.

Table 1. Number of days of bleeding during the various phases of the study (run-in 21/7 cycles)

Duration of withdrawal bleeding (mean ± SD) (n=102)	4.4 ± 1.2 days
Breakthrough bleeding	
During the first 21 day cycle	5.8% (6/103)
During the second 21 day cycle	2.9% (3/103)
During both 21 day cycles	9.7% (10*/103)
Duration (average number of days)	0.5 ± 2.2 days
After first extended contraceptive cycle	
Duration of withdrawal bleeding (mean ± SD) (n=85)	4.9 ± 2.3 days
Breakthrough bleeding	52.8% (47/89)
Range	1–45 days
≥ 10 days	28.1% (25/89)
Mean ± SD of all patients (n=88)	7.0 ± 10.1 days
Mean ± SD of patients reporting breakthrough bleeding (n=47)	13.5 ± 10.5 days
After second extended contraceptive cycle	
Duration of withdrawal bleeding (mean ± SD) (n=68)	4.8 ± 1.6 days
Breakthrough bleeding	43.7% (31/71)
Range	1–49 days
≥ 10 days	18.3% (13/71)
Mean ± SD of all patients (n=71)	5.1 ± 9.5 days
Mean ± SD of patients reporting breakthrough bleeding (n=28)	12.4 ± 11.6 days

* One patient did not specify cycle

Seventy-three patients completed the PGWBI questionnaire. The median (range) global PGWBI scores were not substantially different before and after the extended-use cycles: 78.2 (39.1–96.4) and 77.3 (30.9–96.4) respectively. The scores for the six PGWBI mood states are presented in Table 3.

After the first extended contraceptive cycle, 44.8% of the women were highly satisfied and 28.7% were satisfied with the extended regimen. This high satisfaction rate was slightly less at the completion of the study: 65.5% of the women were either highly satisfied (41.4%) or satisfied (24.1%) with the extended regimen.

DISCUSSION

The present study has shown that the extended 84/7 use of combined oral contraceptives, omitting the hormone-free interval, has a favorable effect on several parameters. According to our results it substantially reduces the frequency of withdrawal bleeding, with a decrease of about one-third in the number of bleeding days compared to the two 21/7 run-in cycles. This regimen also reduced the amount of intermenstrual bleeding.

Bleeding patterns have been addressed in several studies [5,8,14-16], most of which showed no difference between groups, or less bleeding and/or spotting with continuous use of the contraceptives. Coutinho and co-authors [14] reported a mean of 10.7 fewer total bleeding days in the continuous group. Anderson and Hait [15] evaluated bleeding patterns over the entire 364 day study period and reported no significant difference between groups for the mean bleeding plus spotting days, although the continuous arm had fewer bleeding days. Cachrimanidou et al. [16] analyzed the bleeding pattern during the withdrawal week separately and found that it was decreased for the 70 day cycle compared to the 28 day cycle, but that the mean number of bleeding and spotting days was higher in the extended-cycle group. In a recent study, Legro and team [5] did not find any difference in the number of bleeding days between the continuous versus cyclic regimen, although they did observe that there were fewer moderate to heavy bleeding days.

A methodologic limitation of the present study is related to the fact that the two 21/7 cycles preceded the two extended 84/7 oral contraceptive cycles without a "washout period" between the two regimens. As it is well recognized that the number of bleeding days tends to decrease with duration of oral contraceptive use, this may have biased our findings to some extent. However, since our study population of young women was in constant need of a highly effective contraception, and considering the more limited compliance with backup methods (mainly the male condom), we were concerned that a prolonged "washout period" between the two study periods would expose the participants to an unacceptably high risk for unwanted pregnancies. Therefore, we decided that such a "washout period," although fulfilling an apparent methodologic need, would be unfeasible from an ethical point of view.

Dysmenorrhea, heavy bleeding, menorrhagia, abdominal bloating, breast tenderness, irritability and depressive mood were all less common in our study participants who followed the continuous pill regimen. Our search of the literature yielded only one randomized controlled trial that addressed menstrual pain, that of Kwiecien et al. [8] who showed that women in the continuous group are less likely to report menstrual pain. Cachrimanidou et al. [16] reported no improvement in dysmenorrhea.

Few studies have addressed the issue of continuous contraceptive use and other menstruation-associated symptoms. Our study findings showed an improvement in irritability and depression. Cachrimanidou et al. [16] reported a beneficial effect for headaches, but no improvement in any other menstruation-associated symptoms. Legro and colleagues [14] recently demonstrated that the continuous regimen is associated with improved premenstrual but not menstrual or intermenstrual related symptoms compared to a cyclic regi-

Table 2. Symptoms and side effects reported during the various phases of the study

	At enrollment		Start of first extended period		End of first extended period		End of second extended period	
Total no. of women	109		103		86		72	
Intensity of bleeding								
Weak	14	12.84%	24	23.30%	23	26.74%	28	38.89%*
Normal	64	58.72%	70	67.96%	52	60.47%	38	52.78%
Heavy	31	28.44%	9	8.74%	11	12.79%	6	8.33%*
Intermenstrual bleeding								
Yes	87	79.82%	13	12.62%	42	48.84%	31	43.06%*
No	22	20.18%	90	87.38%	44	51.16%	41	56.94%
Dysmenorrhea								
No	33	30.28%	60	58.25%	53	61.63%	48	66.67%*
Light	26	23.85%	36	34.95%	23	26.74%	19	26.39%
Strong	50	45.87%	7	6.80%	10	11.63%	5	6.94%*
Abdominal bloating								
No	48	44.04%	65	63.11%	59	68.60%	57	79.17%*
Light	32	29.36%	25	24.27%	16	18.60%	7	9.72%
Moderate	27	24.77%	12	11.65%	9	10.47%	7	9.72%
Strong	2	1.83%	1	0.97%	2	2.33%	1	1.39%
Breast tenderness								
No	64	58.72%	78	75.73%	65	75.58%	61	84.72%*
Light	26	23.85%	11	10.68%	11	12.79%	6	8.33%
Moderate	15	13.76%	6	5.83%	9	10.47%	5	6.94%
Strong	4	3.67%	8	7.77%	1	1.16%	0	0.00%
Edema								
No	101	92.66%	97	94.17%	80	93.02%	70	97.22%#
Light	5	4.59%	6	5.83%	3	3.49%	0	0.00%
Moderate	3	2.75%	0	0.00%	2	2.33%	2	2.78%
Strong	0	0.00%	0	0.00%	1	1.16%	0	0.00%
Depressive moods								
Never	69	63.30%	77	74.76%	70	81.40%	60	83.33%*
Rare	6	5.50%	10	9.71%	6	6.98%	4	5.56%
Occasionally	17	15.60%	10	9.71%	6	6.98%	6	8.33%
Often	17	15.60%	6	5.83%	4	4.65%	2	2.78%
Irritability								
Never	53	48.62%	68	66.02%	64	74.42%	57	79.17%*
Rare	5	4.59%	12	11.65%	9	10.47%	5	6.94%
Occasionally	21	19.27%	13	12.62%	9	10.47%	4	5.56%
Often	30	27.52%	10	9.71%	4	4.65%	6	8.33%

Statistical significance comparing the incidence of the symptoms and side effects at enrollment compared with the end of the second extended period.

* $P < 0.005$

Not significant

Table 3. Mood state as determined using the Psychological General Well-Being Index before and after the extended use cycles

	The median (range) adjusted PGWBI scores	
	Before extended cycles	**After extended cycles**
Anxiety	80.0 (24.0-100)	76.0 (32.0-100)
Depression	93.3 (46.7-100)	93.3 (46.7-100)
Well-being	65.0 (30.0-100)	70.0 (30.0-100)
Self-control	93.3 (26.7-100)	93.3 (46.7-100)
Global health	86.7 (40.0-100)	86.7 (46.7-100)
Vitality	70.0 (30.0-100)	70.0 (25.0-95.0)
Global	78.2 (39.1-96.4)	77.3 (30.9-96.4)

men. Coffee and associates [17] concluded that a continuous regimen of oral contraceptives diminishes premenstrual-like symptoms measured both by the S&W mood scale and the DSR 17 instrument, with the greatest improvement detected during the sixth month of continuous oral contraceptives.

Although oral contraceptives are thought to have an effect on skin condition [18], 85% of our subjects noticed no change: it reportedly deteriorated in 5% of them and improved in nearly 10%.

The most common reasons given for discontinuation were motivational (i.e., loss of interest, attitude towards study participation, and wishing to conceive). Side effects from the tablets themselves comprised a relatively minor reason for quitting (e.g., bleeding and weight gain – 6.4% each).

Two other important issues were addressed in the present study: weight gain and general well-being. Although it is commonly believed that the initiation of oral contraceptives may be followed by water retention and slight weight gain, no significant change in body weight was observed throughout the study period. We also prospectively evaluated menstrual symptoms following extended use of oral contraceptives and demonstrated that they seemed to improve with continuous dosing and that the user satisfaction rate was high.

It was recently suggested that since quality of life is one of the least explored factors in oral contraceptive users, more studies should investigate the impact of oral contraceptives on quality of life and general well-being in this overall healthy population [20]. The PGWBI is a well-recognized questionnaire of general well-being that was developed to provide an instrument for measuring subjective well-being or psychological distress [13]. It is widely used and well validated [21], having been validated in 32 languages, including Hebrew and Russian, both of which were used in the present study. The PGWBI was chosen as an especially suitable measure of subjective well-being and psychological distress since it covers relevant aspects of the emotional changes linked to hormonal fluctuations, such as depressive mood, anxiety, irritability and lack of energy. Two previous studies reported a beneficial effect on psychological general well-being, as measured by the PGWBI after six cycles of treatment with the same hormone combination used in the present study [19,20]. We assessed the psychological well-being by comparing PGWBI scores before and after the extended use cycles, but found no significant changes in the global, as well as six mood PGWBI scores. In conclusion, the findings of this study show that continuous contraceptive usage has several advantages over the cyclic regimen.

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Capsule

Spatial navigation impairment in patients with transient global ischemia

In rodents, hippocampal CA1 neurons play a pivotal role in the processing of spatial memory. However, the contribution of CA1 neurons in the human hippocampus to spatial memory has been difficult to establish. Bartsch et al. studied a group of patients with transient global ischemia, a condition that lasted for at most a few hours and was followed by complete recovery. The patients were tested on a range of complex neuropsychological instruments, including virtual reality, during the ischemic attack. A striking impairment was observed in simple spatial navigation to a hidden target. The performance of the patients was correlated with the duration of the transient global ischemia, as well as with the size of the CA1 lesions.

Science 2010; 328: 1412

Eitan Israeli

Capsule

First steps in creating an artificial lung

Design of artificial systems that mimic in vivo organs could provide a better alternative for understanding mechanisms underlying physiologic responses than current cell-based models or animal tests. Huh et al. have created a tissue-tissue interface of human-cultured epithelial cells and endothelial cells together, with extracellular matrix in a device that models the alveolar-capillary interface of the human lung. The device mimicked physiologic organ-level functions, including pathogen-induced inflammatory responses and responses to cytokine exposure. Breathing-type movements affected acute pulmonary cell toxicity and proinflammatory activity of widely used nano-particulates.

Science 2010; 328: 1662

Eitan Israeli

Capsule

Viral infection triggers central nervous system autoimmunity via activation of CD8+ T cells expressing dual TCRs

Multiple sclerosis is an inflammatory, demyelinating, central nervous system disease mediated by myelin-specific T cells. Environmental triggers that cause the breakdown of myelin-specific T cell tolerance are unknown. Ji and co-workers found that CD8+ myelin basic protein (MBP)-specific T cell tolerance was broken and autoimmunity was induced by infection with a virus that did not express MBP cross-reactive epitopes and did not depend on bystander activation. Instead, the virus activated T cells expressing dual T cell antigen receptors (TCRs) that were able to recognize both MBP and viral antigens. These results demonstrate the importance of dual TCR-expressing T cells in autoimmunity and suggest a mechanism by which a ubiquitous viral infection could trigger autoimmunity in a subset of infected people, as suggested by the etiology of multiple sclerosis.

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Eitan Israeli

External Fixation for the Treatment of Intra-articular Fractures of the Distal Radius: Short-Term Results

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ABSTRACT: **Background:** There is controversy as to which is the preferred treatment for distal radius intra-articular fractures – anatomic reduction or external fixation.

Objectives: To evaluate the radiologic and functional outcome following external fixation of these fractures.

Methods: Between January 2003 and March 2005, 43 patients with distal radius intra-articular fractures were treated using a mini-external AO device. Follow-up of 38 of the patients included X-rays at 1 week, 6 weeks and 6 months postoperatively. The Visual Analogue Scale was used to assess pain levels, and the Lidstrom criteria scale to evaluate functional outcome and wrist motion. Clinical and radiographic results were correlated.

Results: According to the Lidstrom criteria, the results were excellent in 31%, good in 61% and fair in 5.5%; 2.5% had a poor outcome. The results of the VAS were good. Thirty-five patients gained a good range of wrist movement, but 3 had a markedly reduced range. We found statistical correlation between the radiographic and clinical results, emphasizing the value of good reduction. There was no correlation between fracture type (Frykman score) and radiologic results or clinical results.

Conclusions: External fixation seems to be the preferred method of treatment for distal radius intra-articular fractures, assuming that good reduction can be achieved. The procedure is also quick, the risk of infection is small, and there is little damage to the surrounding tissues.

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KEY WORDS: distal radius fracture, Frykman classification, intra-articular fracture, K-wire

Fractures of the distal radius remain the most common fractures seen in the emergency room, with the majority being treated with plaster of paris cast following closed reduction with local anesthesia. However, other distal radial fractures require surgical management and many treatment methods are available. These can be divided into minimally invasive and invasive. The minimally invasive procedure is closed reduction with Kirschner wire and plaster cast or a mini-external fixator device; the invasive method is open reduction with internal fixation using plates. Despite the frequency of distal radius fractures, only a few studies have assessed the optimal surgical strategies for different fractures [1]. In the last decade many publications have supported the use of external fixation methods [2-4], and in view of the current trend of minimally invasive surgery, we set out to evaluate the radiologic and functional outcome of this method to treat distal radial intra-articular fractures.

VAS = Visual Analogue Scale

PATIENTS AND METHODS

Between January 2003 and March 2005, we used the following protocol for patients presenting at the ER with distal radius intra-articular fractures: a) closed reduction with local anesthesia for all adult patients (over 18 years old); b) once the fracture had stabilized, with X-ray confirmation, the patients were discharged; c) if stabilization could not be obtained, the patients were admitted for surgical treatment.

Surgery was performed on the same day or one day later and consisted of closed reduction and fixation with a mini-external AO device (Synthes, USA), using two threaded rods in the second metacarpal and another two radial rods [Figure 1]. In 20 cases, one or two K-wires were also inserted from the radial styloid to stabilize the fracture. All the rods were 2.7 mm, and the K-wires 1.6 mm. The mini-external device was removed 6 weeks later. A regimen of intensive physiotherapy was implemented immediately after discharge.

We performed surgery in 43 patients with distal radius intra-articular fractures; follow-up was conducted in 38 (22 women and 16 men) whose ages ranged from 23 to 81 years (mean 52.4 ± 18.6 years). All cases were unilateral. The fractures were graded according to the Frykman classification. Five patients were lost to follow-up: four patients had multiple injuries and died, and one patient, a tourist, returned to his country of origin.

ER = emergency room
K-wires = Kirschner wires

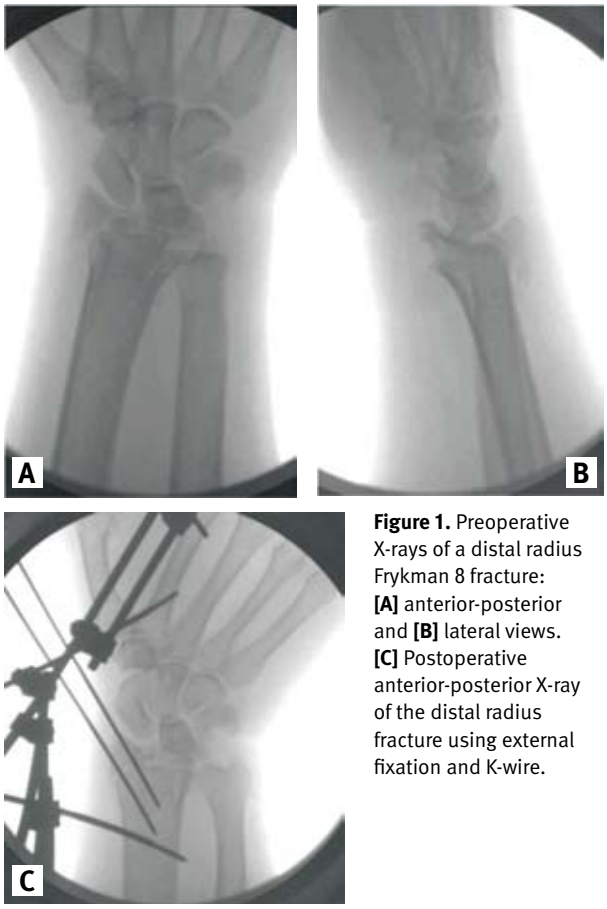

Figure 1. Preoperative X-rays of a distal radius Frykman 8 fracture: **[A]** anterior-posterior and **[B]** lateral views. **[C]** Postoperative anterior-posterior X-ray of the distal radius fracture using external fixation and K-wire.

The follow-up protocol was conducted 1 week, 6 weeks and 6 months postoperatively. The aims of the first two follow-ups were to identify infection and to verify the fracture position with X-ray to dispel the possibility of secondary displacement. The final follow-up, 6 months after surgery, included lateral and anterior-posterior X-rays, assessing the range of wrist movement, quantifying pain by the VAS, and evaluating functional outcome by the Lidstorm criteria [5].

STATISTICAL ANALYSIS

Independent *t*-tests or the Mann-Whitney non-parametric test was used to evaluate differences in continuous demographic and clinical variables between acceptable and non-acceptable volar tilt and radial shortening. Chi-square tests or Fisher exact tests were used to evaluate differences in categorical demographic and clinical variables between acceptable and non-acceptable volar tilt and radial shortening. To evaluate the connection between radial inclination or Lidstrom test and other variables, the Cochran-Armitage test for trend was used for the categorical independent variables and linear regression for continuous independent variables.

RESULTS

We documented pain and functional outcome; alignment was assessed on X-rays. The patients were asked to quantify pain following removal of the mini-external device in order to have a baseline value, and 6 months later. After 6 months, 33 patients (87%) had a VAS > 3. In four patients (11%) the VAS was > 5, and in one patient (2%) with extreme pain it was 9. Regarding functional outcome, 35 patients (92%) had an excellent or good outcome, and 3 (8%) had a fair or poor outcome.

X-RAY EVALUATION

The radial inclination, the volar tilt, and radio-ulna distance were measured on X-ray.

- **Radial inclination:** normal values are between 20° and 22°. In our study 16 (43%) were in the normal range, 15 (39%) were 15–19° and 7 (19%) were 23–25°. Age decreased with increasing radial inclination [Table 1] (from 15–19° to normal to 23–25°, $P < 0.004$). In addition, the Frykman score tended to decrease ($P < 0.10$). The percentage of males, dorsal flexion and ulnar deviation increased with increasing radial inclination ($P < 0.05$, $P < 0.02$ and $P < 0.005$, respectively).
- **Volar tilt:** acceptable values are 0–22° with a mean of 11. In our study 36 (95%) had a volar tilt of 0–22°. Two

Table 1. Demographic and clinical variables by radial inclination

	Radial inclination			
	15–19° (N=15)	Normal (N=16)	23–25° (N=7)	*P* trend
Age (yrs)	62.6 ± 17.9 (median 71)	47.7 ± 19.0 (median 42)	41.1 ± 5.3 (median 44)	0.004
Gender (% males)	26.7%	43.8%	71.4%	0.05
Frykman score	6.0 ± 1.5	5.4 ± 2.0	5.3 ± 1.4	0.10
VAS at 6 wks	5.7 ± 2.3	5.9 ± 1.6	5.1 ± 0.9	NS
VAS at 6 mos	2.3 ± 2.2	2.0 ± 1.5	2.4 ± 0.5	NS
Lindstrom scale				NS
Excellent	5 (33.3%)	6 (37.5%)	1 (14.3%)	
Good	8 (53.3%)	9 (56.2%)	6 (85.7%)	
Poor+fair	2 (13.3%)	1 (6.2%)	0 (0.0%)	
Volar tilt distribution				NS
Acceptable (0–22)	13 (86.7%)	16 (100%)	7 (100%)	
Radial length distribution				NS
2–4 mm shortening	4 (26.7%)	4 (25.0%)	0 (0.0%)	
Dorsal flexion	68.5 ± 12.3	77.6 ± 12.8	80.3 ± 5.9	0.02
Volar flexion	65.4 ± 20.3	70.9 ± 10.2	77.4 ± 4.7	0.07
Ulnar deviation	24.7 ± 13.3	28.9 ± 10.5	41.3 ± 7.5	0.005

Cochran-Armitage test for trend was used for categorical variables and linear regression for continuous variables

(5%) had a dorsal tilt of 2°. Volar tilt tended to increase with increasing radial inclination ($P < 0.07$). Patients with an unacceptable volar tilt [Table 2] were younger ($P < 0.004$) and had a higher Frykman score ($P < 0.0001$) than those with acceptable tilt. In addition, they had lower dorsal flexion ($P < 0.001$) and lower volar flexion ($P < 0.0001$). All patients with unacceptable volar tilt had 2–4 mm shortening of radial length and poor-fair Lindstrom scores, whereas only 16.7% with acceptable volar tilt had 2–4 mm shortening, and 2.8% with acceptable volar tilt had poor-fair Lidstrom scores.

- **Radial length:** normal length is 11 mm, and 2 mm of shortening is acceptable. In our study 29 patients (77%) had less than 2 mm of shortening. In the other 9 patients (23%) the shortening was 2–4 mm. Patients with 2–4 mm radial shortening [Table 3] had a higher Frykman score ($P < 0.02$) and lower volar flexion ($P < 0.04$) than patients with < 2 mm shortening. They tended to have a lower dorsal flexion ($P < 0.08$) and higher VAS at 6 months ($P < 0.07$) than patients with < 2 mm shortening. Patients with 2–4 mm radial shortening had a poorer outcome (Lidstrom) than patients with < 2 mm shortening ($P < 0.001$).

THE LIDSTROM EVALUATION

The percentage of males, the Frykman score and both VAS measures were prognostic factors for the clinical results ($P < 0.003$, $P < 0.0001$ and $P < 0.004$, $P < 0.001$, respectively). Volar tilt and radial length were also prognostic factors for the clinical results ($P < 0.002$ and 0.001, respectively). There was a correlation between the Lidstrom score and the final range of motion of the wrist ($P < 0.001$).

THE FRYKMAN CLASSIFICATION

Ordinal logistic regression was performed to predict the Lidstrom score using the Frykman classification as the main effect and gender as a covariate. For female patients a Frykman score of 3, 4 or 5 predicted excellent results; scores > 5 predicted good results. For male patients a Frykman score of 3 predicted excellent results; scores > 3 predicted good results. Fair and poor results could not be predicted. Ordinal logistic regression was performed to predict radial inclination using the Frykman score as the main effect and gender and age as covariates. Gender was not a significant predictor so it was removed from the model. The Frykman score did not predict radial inclination. Logistic regression was used to predict radial length shortening using the Frykman score as the dependent variable. A Frykman score of 8 predicted > 2 mm shortening. This model could only correctly predict 50% of the patients who had > 2 mm shortening and thus its validity is questionable. With regard to volar tilt, only two patients had an unacceptable tilt; both of these patients had a Frykman score of 8. An additional five patients with a Frykman score of 8 had an acceptable tilt.

Table 2. Demographic and clinical variables by volar tilt

	Volar tilt		
	Acceptable (N=36)	Tilt (N=2)	*P* value
Age (yrs)	53.4 ± 18.6 (median 46)	33.4 ± 3.8 (median 33.5)	0.004*
Gender (% males)	38.9%	100.0%	NS
Frykman score	5.5 ± 1.6	8.0 ± 0.0	0.0001
VAS at 6 wks	5.5 ± 1.6	9.0 ± 1.4	0.005
VAS at 6 mos	1.9 ± 1.2	6.5 ± 3.5	NS**
Lindstrom scale			0.004
Excellent	12 (33.3%)	0 (0.0%)	
Good	23 (63.9%)	0 (0.0%)	
Poor+fair	1 (2.8%)	2 (100.0%)	
Radial inclination			NS
% normal	16 (44.4%)	0 (0.0%)	
Radial length distribution			0.04
2–4 mm shortening	6 (16.7%)	2 (100.0%)	
Dorsal flexion	76.0 ± 10.9	47.5 ± 3.5	0.001
Volar flexion	72.4 ± 10.7	26.0 ± 8.5	0.0001
Ulnar deviation	30.0 ± 11.9	22.0 ± 26.9	NS

Table 3. Demographic and clinical variables by radial length

	Radial length shortening		
	< 2 mm (N=30)	2–4 mm (N=8)	*P* value
Age (yrs)	53.8 ± 17.7 (median 46)	46.9 ± 22.2 (median 39)	NS
Gender (% males)	36.7%	62.5%	NS
Frykman score	5.3 ± 1.6	6.9 ± 1.4	0.02
VAS at 6 wks	5.5 ± 1.7	6.2 ± 2.0	NS
VAS at 6 mos	1.9 ± 1.2	3.1 ± 2.6	0.07*
Lindstrom scale			0.001
Excellent	12 (40.0%)	0 (0.0%)	
Good	18 (60.0%)	5 (62.5%)	
Poor+fair	0 (0.0%)	3 (37.5%)	
Radial inclination			NS
% Normal	12 (40.0%)	4 (50.0%)	
Volvar tilt			0.04
Acceptable	30 (100%)	6 (750.0%)	
Dorsal flexion	77.3 ± 9.1	64.1 ± 17.9	0.08**
Volar flexion	74.0 ± 9.6	54.9 ± 21.4	0.04
Ulnar deviation	31.5 ± 10.9	22.2 ± 16.0	NS*

COMPLICATIONS

There were several complications during our study; 4 patients (10%) had a local superficial pin tract infection treated by oral antibiotics (amoxicillin/clavulanate potassium) for 5 days. One patient (2%) still had pain 6 months later and began treatment for complex regional pain syndrome in the Pain Clinic.

THE ADDITION OF KIRSCHNER WIRE

We compared patients treated with external fixation alone and those who had the addition of K-wire fixation. There were no demographic or X-ray evaluation differences (although there tended to be a difference in volar flexion, $P < 0.07$)). The group that received the addition of K-wire fixation differed in the Frykman score ($P < 0.003$) and VAS at 6 weeks ($P < 0.05$). The VAS was the same at 6 months.

DISCUSSION

The treatment of distal radial fractures is constantly changing. At the end of the 1990s, open reduction and internal fixation by plating was the favored approach, with orthopedic surgeons returning to the principle of ligamentotaxis for fracture reduction [6-8]. This technique for treating unstable distal radius fractures has gained wide acceptance. A number of studies have shown favorable results following external fixation of distal radial fractures [6,8-10]. The addition of K-wire when treating highly unstable fractures was shown to improve the results [7,11-14].

We demonstrated that the addition of K-wire in the less stable fractures elicited an improvement only in VAS score after 6 weeks. In our series, 92% had excellent or good outcomes 6 months postoperatively with 33 patients (86%) having minimal pain (< 3 in the VAS).

On X-ray, the volar tilt was successfully achieved. All but two of the patients (5%) had a volar tilt of up to 16°. The radial length was restored in 77% and excessively shortened by 3–4 mm in 9 patients (23%). We found statistical correlation between the radiographic and the clinical results, emphasizing the importance of achieving good reduction, but there was no correlation between the fracture type (Frykman) and the radiologic or the clinical results.

External fixation seems to be the preferred procedure for treating distal radius intra-articular fractures. The procedure is quick, and the technical learning curve is shorter. The chance of infection is small and less damage occurs in the surrounding tissue compared to open reduction with internal fixation.

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"When you aim for perfection, you discover it's a moving target"

Anonymous

"Thieves respect property. They merely wish the property to become their property that they may more perfectly respect it"

G.K. Chesterton (1874-1936), English writer, whose prolific and diverse output included philosophy, poetry, playwriting, journalism, public lecturing and debating, fantasy and detective fiction

Serum Inflammatory Markers in Overweight Children and Adolescents with Non-Alcoholic Fatty Liver Disease

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ABSTRACT: **Background:** Obesity, a worldwide pandemia, is associated with a large variety of comorbidities, among which is non-alcoholic fatty liver disease. NAFLD is a complex disease that may eventually lead to cirrhosis, posing a high risk for the patient and thus necessitating early diagnosis and treatment.

Objectives: To evaluate the association between ultrasonographically diagnosed non-alcoholic fatty liver disease and the levels of serum inflammatory markers in obese children and adolescents.

Methods: This prospective cohort study was conducted in children and adolescents attending the endocrine obesity clinic in a tertiary care children's hospital in 2001–2003. Blood tests and ultrasound were performed to detect the presence of fatty liver. The severity of fatty liver was determined by measuring the liver/kidney echogenicity ratio (hepatorenal index). Blood tests included complete blood count, liver enzymes, lipid profile, erythrocyte sedimentation rate, high sensitivity C-reactive protein, serum amyloid A, and the degree of erythrocyte adhesiveness/aggregation as measured in peripheral blood slides.

Results: The 30 boys and 34 girls, age 9–21 years, who participated in the study were divided into those who evidenced NAFLD on ultrasound (Group 1, n=37) and those whose liver appeared normal on ultrasound (Group 2, n=24). ESR, hs-CRP, SAA and the degree of erythrocyte adhesiveness/aggregation were compared between the groups. There was no significant association between elevated ESR, the levels of CRP, SAA and/or the degree of erythrocyte adhesiveness/aggregation and the hepatorenal index and NAFLD. The degree of erythrocyte adhesiveness/aggregation correlated with body mass index-standard deviation score in both genders ($P < 0.05$).

Conclusions: Fatty liver itself may not be a cofactor in stimulating inflammatory markers in obese patients. Obese children diagnosed with NAFLD may have simple steatosis and their increased inflammatory markers are therefore compatible with those expected in obesity.

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KEY WORDS: obesity, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, inflammatory markers, steatosis

Non-alcoholic steatohepatitis, a term coined by Ludwig et al. in 1980 [1], is one entity within a spectrum of chronic liver disease related to obesity, hyperinsulinemia, insulin resistance, and liver cell injury from free fatty acid toxicity or other oxidant stress – all related to obesity. The more inclusive term non-alcoholic fatty liver disease represents the entire range, which includes simple hepatic steatosis without inflammation, NASH, and the resulting cirrhosis. Most individuals with simple steatosis do not develop cirrhosis. In contrast, up to 20% of adults with NASH ultimately develop cirrhosis, with some eventually developing chronic liver failure and requiring liver transplantation [2]. To date, the gold standard for diagnosing NAFLD and differentiating among simple steatosis, NASH and cirrhosis is liver biopsy.

The pathogenesis of NAFLD is not completely understood. Among the factors thought to be involved are free fatty acid accumulation in the liver, hyperinsulinemia, inflammatory cytokines (such as tumor necrosis factor-alpha), mitochondrial damage, and free radicals that cause significant oxidative stress [3]. NAFLD may also be a part of the metabolic syndrome [3,4].

Few studies have explored a link between NAFLD and the levels of serum inflammatory markers. In one investigation, 18 adult patients with histologically diagnosed NASH had elevated levels of C-reactive protein and other serum inflammatory markers, compared with 16 healthy individuals [5]. The aim of the current work was to examine the association between the

NAFLD = non-alcoholic fatty liver disease
ESR = erythrocyte sedimentation rate
Hs-CRP = high sensitivity C-reactive protein
SAA = serum amyloid A
NASH = non-alcoholic steatohepatitis

presence of fatty liver (evaluated by liver ultrasound) and serum levels of inflammatory markers in children and adolescents. We reasoned that since obesity itself is associated with low grade systemic inflammation, we may detect even higher levels of serum inflammatory markers in association with pediatric NAFLD, allowing earlier diagnosis and early, aggressive treatment.

PATIENTS AND METHODS

The study group consisted of 64 children and adolescents with body mass index > 85th percentile for age and gender who were attending an endocrine obesity clinic (Schneider Children Hospital) during the period 2001–2003. Excluded were those who drank alcoholic beverages or used medications that can alter liver function tests. In order to compare biochemical and inflammatory marker levels the study cohort was divided into groups: children with fatty liver grade 1 or 2 (group 1), and normal liver grade 0 (grade 2). Each group was further divided into two subgroups according to serum transaminase levels: patients with normal transaminase levels comprised the first subgroup, and those with abnormal levels the second (i.e., alanine aminotransferase, aspartate aminotransferase and/or gamma-glutamyl transpeptidase > 40 mg/L).

For each subject, BMI was calculated using the equation [weight (kg) / height (m)^2], and BMI-standard deviation score was calculated using the equation [(Measured BMI - Expected BMI) / SD], allowing standardization of BMI with gender and age.

LABORATORY STUDIES

Blood tests included complete blood count, liver enzymes, lipid profile, glucose, insulin, glycated hemoglobin, thyroid hormones, vitamin E and inflammatory markers, which included assessment of erythrocyte sedimentation rate, high sensitivity C-reactive protein, serum amyloid A and the degree of erythrocyte aggregation as measured by slide image analysis. The latter has been described in detail elsewhere [21,22].

Elevated liver enzymes were defined as values of ALT and/or AST and/or GGT ≥ 40 mg/L. Hyperlipidemia was defined as low density lipoprotein levels > 130 mg/dl and/or high density lipoprotein-cholesterol levels < 35 mg/dl and/or triglyceride levels > 200 mg/dl. Insulin resistance was calculated by the homeostasis model assessment of insulin resistance and defined as a HOMA value > 2 [10]. HOMA levels were transformed to the natural log to normalize their distribution. Blood tests were performed to rule out any known etiology for fatty liver, including serology assays for hepatitis B and C viruses, tissue transglutaminase and ceruloplasmin level.

Serum glucose was measured by the glucose oxidase colorimetric method using an automated analyzer (Hitachi 917, Roche Diagnostics, USA), and total cholesterol, triglycerides, and HDL-cholesterol concentrations were measured by an enzymatic colorimetric method on an automated analyzer (Hitachi 904, Roche). Serum insulin concentrations were measured by an immunometric assay with the IMMULITE 2000 Analyzer (DPC, Los Angeles, CA, USA). HbA1c levels were measured by the turbidimetric inhibition immunoassay (Hitachi 911, Roche). Cross-sectional analysis was performed to evaluate the association between the biochemical laboratory values and the ultrasound results.

ULTRASONOGRAPHIC STUDY

An ultrasound examination was performed to identify NAFLD. All tests were performed with a single probe by one experienced radiologist, thus minimizing inter- and intra-observer variability. Semi-quantification of the severity of fatty liver using ultrasound is based on calculating the difference between the echo densities of the liver and the right kidney. Fatty liver was recognized by a bright hepatic echo pattern (hyperechogenicity) and thus easily identified as compared to the renal cortex, which has roughly similar echogenicity to normal fat-free liver. This method was found to have sensitivity and specificity of 89% and 93%, respectively [6]. The degree of fatty infiltration in the liver was quantified by using the equation: liver echo amplitude / renal echo amplitude, whereby the hepatorenal index was defined. The hepatorenal index of normal fat-free liver is about 1 In fatty liver; the severity of the liver steatosis was graded as follows: Grade I (mild fatty liver) was defined by a hepatorenal index of 1.5–2, and Grade II (severe fatty liver) by a hepatorenal index ≥ 2.

INFLAMMATION MARKERS STUDY

CRP and SAA were measured using particle-enhanced immunonephelometry (BN prospec system, Dade Behring, Germany). CRP was measured using a high sensitivity CRP assay (reference interval: < 1.69 mg/L in 90% and < 2.87 mg/L in 95% of healthy individuals), and SAA was measured using latex SAA (reference interval < 6.4 mg/L in individuals with normal serum CRP). Blood slides were prepared from blood drawn into a syringe containing sodium citrate 3.8%, and scanned by an image analyzer. For each blood slide, several parameters were measured using the image analyzer, including vacuum ratio and erythrocyte percent. These parameters represent the degree of erythrocyte aggregation, and thus the degree of inflammation.

The study protocol was approved by the local Helsinki Committee. A signed informed consent was obtained from all the patients or their parents.

BMI = body mass index
ALT = alanine aminotransferase
AST = alanine aminotransferase
GGT = gamma-glutamyl transpeptidase
HOMA = homeostasis model assessment of insulin resistance
HDL = high density lipoprotein
HbA1c = glycated hemoglobin

STATISTICAL ANALYSIS

All data were summarized and displayed as mean ± SD for continuous variables and as number of patients plus the percentage in each group for categorical variables. The cross-tabulations and descriptive procedures were used to produce frequencies of categorical variables and means ± SD of continuous variables. We used a logarithmic transformation for variables that have a non-normal distribution (e.g., the hs-CRP and the SAA), thus converting them to normal distribution for all statistical procedures, such as *t*-tests and correlations. Each result expressed as hs-CRP or SAA is a back-transformed geometric mean and standard deviation. The one-sample Kolmogorov-Smirnov test was used to test for normal distribution.

Student's *t*-test for independent samples was used for all normally distributed continuous variables when comparing two categories (e.g., gender), while the one-way ANOVA was used to compare the various parameters between the groups when comparing more than two categories. The pair-wise comparison between categories was done after performing the Levene test for homogeneity of the variance: the Hochberg's multiple comparison technique was applied when the Levene test was not significant, while the Dunnett's T3 test was applied when it was significant. Chi-square phi and Cramer's V statistics were used for assessing the overall significance across all the diagnosis groups for all categorical variables.

The level of significance used for all of the above analyses was two-tailed $P < 0.05$. The SPSS statistical package was used to perform all statistical evaluation (SSPS Inc., Chicago, IL, USA).

RESULTS

The study group consisted of 30 boys and 34 girls. Their mean age was 14.9 ± 2.8 years (range 9–21), their mean BMI was 34.6 ± 5.5, and their mean BMI-SDS 3.3 ± 1. The BMI was > 95th percentile in most of them (92%) and between the 85th and 95th percentiles in the rest. Around three-quarters (72.5%) of the patients were at advanced stages of puberty (Tanner IV-V). Familial obesity was ubiquitous, and the prevalence of type 2 diabetes and ischemic heart disease in their first- and second-degree relatives was 63% and 37%, respectively. There were no significant gender differences for age, BMI and BMI-SDS.

ULTRASOUND DATA

Twenty-seven patients had grade 0, 19 had grade I and 18 had grade II hepatic steatosis according to the ultrasonographically calculated hepatorenal index. The average BMI-SDS in the group of children with fatty liver (mild or severe) was significantly higher than the average BMI-SDS in those with normal liver [Figure 1].

BMI-SDS = body mass index-standard deviation score

Figure 1. Significant difference between the degree of fatty liver and body mass index/standard deviation score (BMI-SDS) in the groups with severe fatty liver and normal liver ($P < 0.01$) and in the groups with mild fatty liver and normal liver ($P < 0.01$)

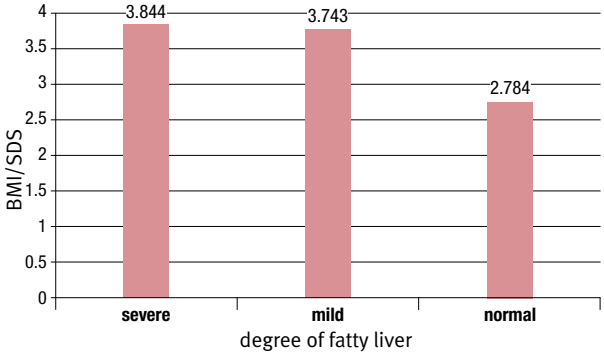
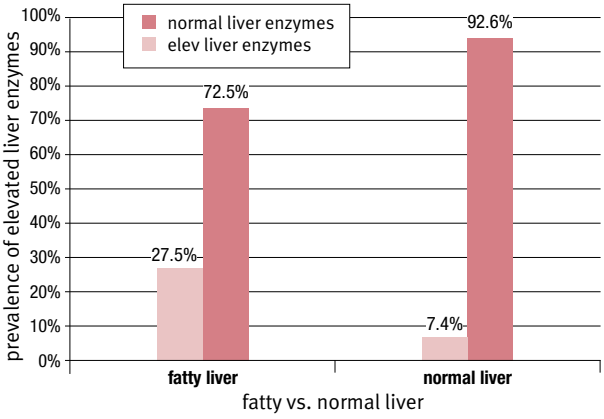


Figure 2. Relation between the degree of fatty liver and the prevalence of elevated liver enzymes. There was a significant difference between the prevalence of elevated liver enzymes in children with fatty liver and in children with normal liver ($P < 0.059$)



BIOCHEMICAL RESULTS

Hyperlipidemia was present in 47 patients (73%); 50 (78%) had insulin resistance, and 12 (19%) had elevated liver enzymes (ALT and AST). The ALT/AST ratio was > 1 in all 12 patients whose AST and ALT values were elevated. The ALT levels averaged 24.3 ± 15.6 mg/dl, and AST 21 ± 8.9 mg/dl. The AST/ALT ratio was 0.864. Alkaline phosphatase levels were mildly elevated, consistent with the elevations found in growing adolescents. Bilirubin levels were normal in all patients and the mean leptin level was 66.2 ± 31.3 ng/ml (range 16.4–167 ng/ml). The prevalence of elevated liver enzymes between the groups with and without fatty liver was significantly different [Figure 2].

INFLAMMATORY MARKERS

The average levels were as follows: ESR 18.97 ± 14.96 mm/hr, white blood cells 8.11 ± 2.26 x 10^3/µl, hemoglobin 13.15

± 1.2 g/dl, CRP 4.2 ± 2.7 mg/L (range 0.45–46.7 mg/L), and SAA 6.2 ± 2.3 mg/L (range 1.37–92.7) [Table 1].

CORRELATION OF ESR, ELEVATED LIVER ENZYMES AND ULTRASOUND FINDINGS

There was no significant difference in ESR between subjects with normal ultrasound findings and those with ultrasound findings of fatty liver and elevated or non-elevated liver enzymes. The average ESR was 16.5 ± 11.5 mm/hr in patients with normal ultrasound findings (grade 0 hepatic steatosis), and 18.8 ± 14.1 mm/hr in patients with ultrasound findings of NAFLD (grades I and II hepatic steatosis). There was no significant difference between the two groups. Further dividing the latter group into subgroups of patients with grade I hepatic steatosis and patients with grade II hepatic steatosis also showed no significant difference in the ESR between them.

CORRELATION OF HEPATORENAL INDEX AND INFLAMMATORY MARKERS

There was no significant correlation between the hepatorenal index and the levels of the tested inflammatory markers (CRP, SAA, EP and VR).

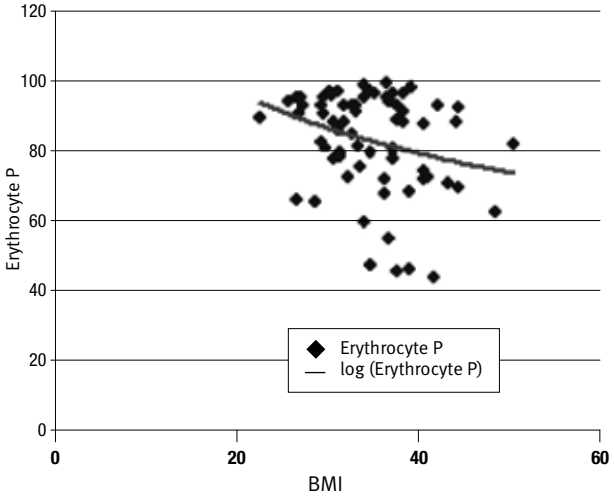
CORRELATION OF BMI AND HISTOLOGIC INFLAMMATORY MARKERS

An inverse correlation was found between the BMI and EP in the female patients (N=34, $P < 0.05$) [Figure 3]. A direct correlation was also found between the BMI, BMI-SDS and VR in the male patients (N=30, $P < 0.05$). These findings demonstrate the correlation between BMI and the degree of inflammation as represented by the peripheral blood slides.

Table 1. Inflammatory markers in the study group

	Hemoglobin (mg/L)	Leukocytes (10^9/L)	ESR (mm/hr)	hs-CRP (mg/L)	SAA (mg/L)	Erythrocyte percent	Vacuum ratio
N	64	64	64	64	64	64	64
Average	13.15	8.11	18.97	4.2	6.16	83.29	8.44
Median	12.9	8.1	14.5	4.4	5.42	88.53	5.69
Standard deviation	1.23	2.26	14.26	2.7	2.32	14.16	7.99
Maximum	17.6	14.3	68	46.7	92.7	99.8	46.13
Minimum	10.9	3.1	2	0.45	1.37	44.52	1.89

Figure 3. Body mass index (BMI) and erythrocyte percent (Erythrocyte P) was inversely correlated in females ($P < 0.05$)



DISCUSSION

Obesity is a global medical problem in the general population. The pediatric population is not spared. It is reported that around 15% of children between age 6 and 19 are above the 95th percentile of BMI. According to previous reports, there is a clear suboptimal diagnosis of obesity and its complications in children visiting clinics in Israel [10,11], emphasizing the need for early evaluation, diagnosis and treatment. Furthermore, these children are at high risk of developing cardiovascular disease in early adulthood; this risk can be assessed and treated early in their life [12]. There is a clear association between obesity and NAFLD: 85% of children with NAFLD are obese [13]. The prevalence of NAFLD, diagnosed by detecting hyper-echogenicity of the liver on ultrasound, was found to be 2.6% in normal-weight children and an alarming 22.5–52.8% in obese children [14,15]. The true prevalence of NAFLD in the pediatric population may be underestimated. A recent retrospective review of 742 autopsies of children aged 2–19 years noted the presence of fatty liver in 13% of all subjects and in 38% of the obese children among them [16,17]. Given the high prevalence of fatty liver among obese children, it is crucial to determine the role of fatty liver in the overall morbidity of those children. Demonstrating high levels of inflammatory markers will emphasize the risk posed by fatty liver and the importance of early and aggressive treatment.

Like adults, most children are asymptomatic. In those who are symptomatic, the most common complaint is right upper quadrant pain or chronic periumbilical abdominal pain [13]. Findings on physical examination may include obesity, hepatomegaly, acanthosis nigricans, or splenomegaly (rare). Common comorbidities include hyperlipidemia, hypertension, insulin resistance and diabetes mellitus. These conditions, along with NASH, are considered part of the metabolic syndrome [13].

Mildly elevated levels of serum ALT may be found in children with NAFLD, with some having levels 10 times the

EP = erythrocyte percent
VR = vacuum ratio

normal [1]. Among obese children and adolescents, the percentage of those with elevated levels of serum transaminases ranges between 12 and 25% [13]. Serum GGT and alkaline phosphatase may be mildly elevated as well. Ultrasound evaluation of the liver in patients with NAFLD may show diffuse hyper-echogenicity compared to the kidneys. The sensitivity and specificity of ultrasound are 89% and 93% as a diagnostic tool for fatty liver, and 89% and 77% respectively to detect liver fibrosis [6]. Ultrasound has many drawbacks as a diagnostic tool because it is operator dependent, and in very obese children the subcutaneous fat can interfere with the scanning. However, in this study there was no difficulty performing the scan.

Accumulating data show high levels of serum inflammatory markers in obese individuals. Ongoing systemic inflammation in obese adults was evidenced by high levels of CRP and fibrinogen [18,19]. Similar findings were found in children [20]. To date, few studies have investigated the correlation between NAFLD and the levels of serum inflammatory markers, and all were conducted in adults [5]. The general consensus of these studies was that high serum inflammatory markers can be found in patients with NAFLD.

The inflammatory markers include CRP, fibrinogen, SAA, and inflammatory cytokines interleukin-6 and TNFα. High levels of CRP in the serum are known to be related to increased risk for cardiovascular morbidity, including the early development of atherosclerosis [21,22]. The degree of erythrocyte adhesiveness/aggregation in peripheral blood slides is another inflammation marker found to be related to obesity [24]. The correlation between the degree of erythrocyte adhesiveness/aggregation is directly related to the degree of systemic inflammation and to cardiovascular risk [25]. Given the possible relation between NAFLD and elevated levels of serum inflammatory markers, early diagnosis of NAFLD and aggressive treatment may play an important role in preventing both the progression of NAFLD into severe hepatic inflammation or cirrhosis and the early development of atherosclerosis. Furthermore, using a panel of serum inflammatory markers in conjunction with serum transaminases and ultrasound findings may replace the need for biopsy to establish the diagnosis of NASH. Treatment with statins, which act as anti-inflammatory agents in addition to lowering LDL, was recently associated with a significant reduction in CRP serum levels [23]. It therefore follows that earlier treatment with statins against the background of NAFLD may reduce even further the risk of cardiovascular morbidity.

Ultrasonically detected fatty liver did not correlate with the levels of inflammatory markers. This may be explained by several factors: a) affected children suffer from simple fatty liver and not NASH (defined as "an inflammation of the liver"); b) obese children already have increased levels of serum inflammatory markers that may mask the small increment possibly caused by NASH itself; c) other factors, such as toxicity of triglycerides, may contribute to the development of NASH, thus weakening the correlation between "pure" inflammatory markers and NASH. In contrast, there is evidence of a significant correlation between BMI and inflammatory markers, both in serum (CRP, SAA) and in blood smears, using the image analyzer of blood smears.

It is important to emphasize that the ultrasonographic evaluation of fatty liver in this study does not differentiate well between simple steatosis and NASH. As such, further research is required, including histologic study, to correlate between the different stages of NAFLD and the levels of serum inflammatory markers in the pediatric population.

TNFα = tumor necrosis factor-alpha
LDL = low density lipoprotein

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Capsule

The immune system and the gut flora

The mammalian gut is colonized by many non-pathogenic, commensal microbes. In order to prevent the body from mounting inappropriate immune responses to these microbes, plasma cells in the gut produce large amounts of immunoglobulin A (IgA) specific for commensal bacteria. Because of the difficulties of uncoupling IgA production from microbial colonization, how commensal bacteria shape the gut IgA response is not well understood. Hapfelmeier et al. devised a way to get around this problem by developing a reversible system of gut bacterial colonization in mice. Commensal-specific IgA responses were able to persist for long periods in the absence of microbial colonization and required the presence of high microbial loads in the gut for their induction. IgA responses upon bacterial re-exposure did not resemble the synergistic prime-boost effect seen in classical immunologic memory responses but rather exhibited an additive effect that matched the current bacterial content present in the gut. The body thus constantly adapts the commensal-specific immune response to the microbial species present in the gut, which contrasts with the systemic immune response that persists in the absence of pathogenic microbes.

Science 2010; 328: 1705

Eitan Israeli

Capsule

Oxytocin and intergroup parochial altruist conflict

Human society is organized into groups, such as those based on nationality or religion, which can lead to intergroup conflicts, with sometimes devastating consequences. Intergroup conflict engages a human behavior termed parochial altruism: For example, a soldier who fights against the enemy at risk to themselves to protect their country is a parochial altruist. De Dreu et al. have discovered a role for oxytocin, a neuropeptide produced in the hypothalamus, in regulating parochial altruism during human intergroup competition and conflict. Oxytocin is already known to play a role in trusting behavior, and naturally occurring genetic variants of the oxytocin receptor exist within the human population. Administration of oxytocin modulated defense-related aggression toward competing groups but did not affect unprovoked, hateful behavior. Thus, there may be a neurobiologic basis for intergroup conflict in humans.

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Eitan Israeli

"It must be borne in mind that the tragedy of life does not lie in not reaching your goal. The tragedy of life lies in having no goal to reach"

Benjamin E. Mays (1894-1984), U.S. Black minister, educator, scholar, social activist and college president. An articulate and outspoken critic of segregation before the rise of the modern civil rights movement in the United States, he was a significant mentor to civil rights leader Martin Luther King Jr.

Parotid Mass as Presenting Symptom of Lymphoma*

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ABSTRACT: **Background:** Lymphomas arising from the parotid gland are an uncommon entity, said to account for only 0.6–5% of tumors or tumor-like lesions of the parotid, and are therefore commonly overlooked. This misdiagnosis often leads to unnecessary diagnostic procedures, delaying the initiation of proper treatment.

Objectives: To examine the clinical, diagnostic, therapeutic and survival data of patients with this disease.

Methods: We retrospectively reviewed our experience with patients diagnosed and treated for parotid lymphoma in our medical center during the period 1998–2008.

Results: The 13 patients in the series were aged 42–83. Twelve had non-Hodgkin's lymphoma and 1 had Hodgkin's lymphoma. In eight, parotid mass was the first manifestation of the disease, while in five who were in clinical remission its reoccurrence was first manifested in the parotid gland. Mean survival was 6.3 years

Conclusions: Since parotid lymphoma is uncommon, it is often overlooked in the differential diagnosis. Methods of diagnosing and treating parotid lymphoma are different from those of other parotid pathologies. A high index of suspicion is warranted in order to provide a quick and efficient diagnosis and treatment without subjecting the patient to unnecessary tests and procedures.

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KEY WORDS: parotid, lymphoma, salivary gland, B symptoms, fine needle aspiration

Primary lymphoma of the parotid is not often encountered in everyday practice. Reports describing series of patients and the preferred means of diagnosis and treatment are scarce [Table 1]. We review our experience with a series of 13 patients diagnosed with primary lymphoma of the parotid.

PATIENTS AND METHODS

We conducted a retrospective chart review of 13 patients aged 43 to 83 years with parotid mass who were diagnosed with parotid lymphoma and treated in our tertiary medical center between the years 1998 and 2008. In eight of them the mass was related to primary parotid lymphoma, while in five it was the first manifestation of its recurrence.

At the time of data accumulation there was no requirement for approval by an Institutional Review Board.

RESULTS

All patients [Table 2] in our series had a painless mass in their parotid gland [Figure 1]. None had B symptoms (i.e., fever, night sweats or weight loss). Mean age at presentation was 63.5 (age range 43–83 years). The male/female ratio was 1.6. One patient presented with bilaterally enlarged parotid glands; the rest had unilateral enlargement. Five patients had a previous diagnosis of lymphoma but were in clinical remission (ranging from 18 months to 10 years). When the patients were thoroughly examined for other sites of involvement, five had systemic findings on positron emission tomography–computed tomography. Clinically undetected submandibular lymph node involvement was found in two patients, retroperitoneal involvement in four, axillary in two (in one of whom it was bilateral) and inguinal nodes in one. Diagnosis was made in

Table 1. Series of parotid lymphoma in the literature

Author, year [ref]	Patients with primary parotid lymphoma	Treatment	Survival
Freedman, 1971 [19]	8	NA	NA
Hyman & Wolff, 1976 [13]	30	Local excision + Rx	NA
Colby & Dorfman, 1979 [20]	42	NA	NA
Schmid et al., 1982 [21]	21	NA	83% 5 yrs
Gleeson et al., 1986 [22]	21	Local excision + Rx	49 mos
Schusterman et al., 1988 [9]	19	NA	NA
Takahashi et al., 1990 [23]	14	NA	NA
Mehle et al., 1993 [5]	18	Cx/Rx	83.4 mos
Barnes et al., 1998 [11]	33	Cx/Rx	75% 2 yrs
von Stritzky et al., 1998 [8]	7	Cx/Rx	85% 66 mos
Allen et al., 1999 [24]	12	NA	NA
Tiplady et al., 2004 [1]	136	NA	90 mos
Dunn et al., 2004 [25]	13	Cx/Rx	94.7% 5 yrs

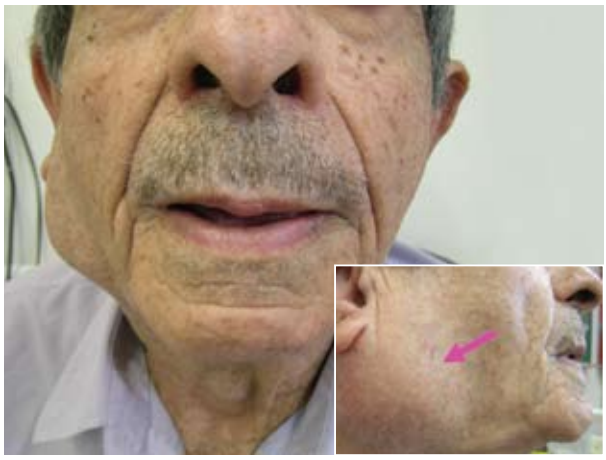
Cx = chemotherapy, Rx = radiotherapy

*Presented at the Second International Congress on Salivary Gland Diseases, Pittsburgh, Pennsylvania, USA, 19-21 October 2007

Table 2. Demographics, diagnostic procedures and CT findings

Patient #	Gender/ Age (yrs)	Extraparotid involvement on CT	Fine needle aspiration	Diagnostic procedure
1	M/54	Yes	No	Biopsy
2	F/43	No	No	Partial parotidectomy
3	M/46	No	Yes (non-diagnostic)	Biopsy
4	F/76	No	No	Biopsy
5	M/53	Yes	No	Biopsy
6	F/70	No	No	Partial parotidectomy
7	M/75	No	Yes (non-diagnostic)	Biopsy
8	M/83	Yes	Yes (non-diagnostic)	Biopsy
9	M/76	Yes	Yes (non-diagnostic)	Parotidectomy
10	M/54	Yes	Yes (non-diagnostic)	Biopsy
11	F/50	Yes	Yes (non-diagnostic)	Biopsy
12	M/73	No	Yes (non-diagnostic)	Biopsy
13	F/72	No	No	Parotidectomy

Figure 1. Periauricular mass in a 76 year old man with parotid lymphoma



all patients on the basis of histologic and immunohistologic findings consistent with lymphoma. Tissue was obtained by open biopsy in nine of the patients and by parotidectomy in four. Seven had previously had fine needle aspiration, which was non-diagnostic. None of our patients suffered any permanent injury to the facial nerve; one had transient unilateral facial nerve weakness with complete spontaneous resolution 2 months after surgery. The histologic examination results are presented in Table 3. All patients but one were treated in our hemato-oncology department and records of this patient were not available. Five patients were graded as stage I disease, one stage II, two were stage III and two were stage III-IV. For three of the patients there were no data regarding clinical staging (in two because of the urgency to start treatment and the third was

Table 3. Hematologic data and survival

Patient #	Type of B cell lymphoma	Stage on presentation	Initial treatment	Radiation	Follow-up (yrs)	Survival
1	Mixed cell	III	Chlorambucil	Yes	9	Alive
2	Marginal cell	I	CHOP	Yes	10	Alive
3	Large cell	I	CHOP	Yes	9	Alive
4	Large cell	NA	COP	No	3	3 yrs
5	Follicular	III	NA	No	10	Alive
6	Follicular	III/IV	Chlorambucil	No	7	Alive
7	T cell rich	NA	CHOP	No	9	9 yrs
8	Large cell	II	R-CHOP	No	4	Alive
9	B cell lymphoma	NA	NA	NA	3	Alive
10	Low grade	III/IV	R-CHOP	Yes	2	Alive
11	Mixed cell	Ia	ABVD	Yes	3	Alive
12	Large cell	I	CHOP	Yes	1	Alive
13	Follicular	I	No	No	1	Alive

CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone
R-CHOP = CHOP + rituximab
ABVD = adriamycin, bleomycin, vinblastine, dacarbazine
NA = not available

treated elsewhere). Treatment was mainly chemotherapy, with five patients having the CHOP protocol, two chlorambucil, one COP and one ABVD. Six also had radiation therapy. Only two patients died; their deaths were disease related. Mean survival was 6.3 years [Table 2].

DISCUSSION

Salivary gland tumors are responsible for only 3–4% of primary head and neck tumors. The parotid gland is by far the most common site for salivary gland malignancies, accounting for 70–80% of all salivary gland malignancies [1,2], with an estimated incidence of 1.2 per 100,000 persons [3]. The most common malignancy of the parotid is mucoepidermoid carcinoma followed by adenoidcystic carcinoma [2]. Malignancies account for only 20% of parotid-related tumors, yet the clinical manifestations in 80% of tumors diagnosed as malignant resemble those encountered in benign tumors [4].

Lymphoma is a very common malignancy and the second most common neoplasm of the head and neck after squamous cell carcinoma. Most of the non-Hodgkin's lymphomas arise primarily in the lymph nodes (71.9%), while only 29.1% are primarily extranodal. Primary parotid lymphoma accounts for only 0.87% of all NHL cases (3.1% of extranodal NHLs) [5-7]. This type of malignancy constitutes 0.2–0.8% of malignant tumors in the parotid gland [8], although there is concern in the literature that the prevalence of this rare malignancy

NHL = non-Hodgkin's lymphomas

has risen in recent decades [9,10]. The population known to have a much higher incidence of primary lymphoma of the parotid is the one with autoimmune diseases, the strongest correlation being with Sjögren syndrome [11,12].

There is controversy in the literature regarding whether the parotid is truly primarily involved or whether it arises in intraglandular lymph nodes [3]. Hyman and Wolff [13] proposed criteria for the diagnosis of primary parotid lymphoma: a) involvement of the salivary gland as the first clinical manifestation of disease; b) histologic proof that lymphosarcoma involves the salivary gland parenchyma, rather then being confined to soft tissue or a lymph node in the area; c) architectural and cytologic confirmation of the malignant nature of the infiltrate. For the purpose of this article we used these criteria and considered the lymphoma as a truly primary lymphoma regardless of whether it manifested first at the parotid gland or was the first manifestation of a reoccurrence in a patient previously diagnosed with lymphoma in remission.

Parotid lymphoma most commonly presents as a painless mass indistinguishable from other non-malignant or other more common epithelial tumors. This explains why this diagnosis is commonly overlooked and patients are often subjected to unnecessary procedures and a delay in diagnosis. In most cases the facial nerve is not jeopardized [3,11]. Diagnosing parotid lymphoma can be a difficult task, as evidenced by the unnecessary tests conducted before correct diagnosis is made [14]. Both from our experience and from the review of the literature, FNA is not diagnostic and therefore should be avoided whenever a high index of suspicion for lymphoma arises in the differential diagnosis (e.g., B symptoms, enlarged lymph nodes, or a previous history of lymphoma) [3,15,17]. In these cases, the most accessible lymph node should be biopsied. In the case of an isolated parotid mass, differentiating it is almost impossible and FNA is usually performed. CT scan may add information regarding the malignant nature of the disease, with signs such as irregular borders and extraparotid extension. Currently, there are still no pathognomonic findings indicative of lymphoma on CT [18]. The procedure of choice for the diagnosis of lymphoma in the parotid gland should be core biopsy.

CONCLUSIONS

Although primary parotid lymphoma is a rare entity, this diagnosis should always be kept in mind when a patient presents with a non-tender mass in this gland. The importance of considering this diagnosis cannot be overemphasized since it may save the patient from undergoing unnecessary diagnostic procedures, thereby prompting the appropriate medical treatment. The prognosis for a well-evaluated and appropriately treated patient with lymphoma presenting in the parotid gland is excellent.

FNA = fine needle aspiration

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Interleukin-6 and N-Terminal Pro-Brain Natriuretic Peptide Cord Blood Levels in Premature Infants: Correlations with Perinatal Variables

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ABSTRACT: **Background:** Elevated cord blood levels of interleukin-6 and N-terminal pro-brain natriuretic peptide were associated with neonatal complications; however, simultaneously obtained values have not been compared to date.

Objectives: To study the association of cord blood levels of IL-6 and NT-proBNP with perinatal variables of premature infants and examine the relationship between the obtained values.

Methods: Cord blood IL-6 (89 samples) and NT-proBNP (66 samples) levels obtained from infants delivered before 32 weeks of gestation were analyzed for associations with perinatal variables and possible correlation between both samples.

Results: Lower gestational age, no antenatal exogenous steroids, low Apgar scores at 1 minute and delivery at a high birth order, were all associated with more infants having elevated IL-6 levels (P = 0.02, P = 0.03, P = 0.03 and P = 0.001, respectively). None of the infants with necrotizing enterocolitis (n=6) had high IL-6 levels (P = 0.01). Increased NT-proBNP levels were associated with low Apgar scores at 1 minute (P = 0.01) and the presence of clinical chorioamnionitis (P = 0.06). Controlling for gestational age, a weak positive correlation was demonstrated between IL-6 and NT-proBNP levels in infants of 24–27 weeks gestational age (R^2 = 0.151, P = 0.08), but not among the more mature infants.

Conclusions: Although both IL-6 and NT-proBNP values were significantly associated with low 1 minute Apgar scores, our results do not support utilization of these cord blood levels as the sole tool to predict neonatal outcome.

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KEY WORDS: cord blood, fetal inflammatory response syndrome, interleukin-6, N-terminal pro-brain natriuretic peptide, prematurity

The inflammatory reaction to intrauterine infection is associated with preterm labor and delivery and was found to correlate with intraamniotic and cord blood cytokine levels and with illness severity in the newborn. The fetal participation in this process was designated the "fetal inflammatory response syndrome," a condition indicated by histologic inflammatory changes in the cord vessels (funisitis) and by cord blood interleukin-6 levels ≥ 11 pg/ml [1,2]. Most studies have focused on the neurologic (intraventricular hemorrhage, periventricular leukomalacia, cerebral palsy) and pulmonary (respiratory distress syndrome, bronchopulmonary dysplasia) consequences of FIRS [3-6]. However, evidence suggests the involvement of other fetal target organs in the process as well, including the hematopoietic system, adrenal glands, heart, skin and kidneys [7].

With reference to the cardiovascular system, Yanowitz and colleagues [8] found that chorioamnionitis and elevated cord blood IL-6 concentrations are associated with decreased blood pressure in premature newborns. Romero et al. [9] observed changes in fetal cardiac function consistent with increased left ventricular compliance observed in preterm premature rupture of membranes, particularly in cases with intraamniotic infection. The role of proinflammatory cytokines in the pathogenesis of myocardial dysfunction was thus suggested.

N-terminal pro-brain natriuretic peptide, a segment of the B type natriuretic peptide prohormone, is a sensitive marker of left ventricular dysfunction. The peptide is produced and secreted by the cardiac myocytes in response to left ventricular stretch and compromise [10]. Circulating peptide levels correlate inversely with left ventricular function in adults and children [11-13]. Previous studies demonstrated a rise in blood NT-proBNP level after birth with subsequent decline parallel to the retreat of pulmonary pressure [13,14]. Umbilical cord blood levels were high following fetal distress and in venous samples of newborn infants with persistent pulmonary hypertension [15,16].

IL-6 = interleukin-6
NT-proBNP = N-terminal pro-brain natriuretic peptide

FIRS = fetal inflammatory response syndrome

Since both IL-6 and NT-proBNP levels at delivery appear to have prognostic implications for premature newborns, we decided to examine their relationship with perinatal variables. Also, since both agents were implicated in neonatal cardiac dysfunction, we examined whether there was a correlation between simultaneously obtained levels.

PATIENTS AND METHODS

All premature infants delivered in the two Hadassah hospitals (Ein Kerem and Mt. Scopus campuses) in Jerusalem before 32 weeks gestation and admitted to our nurseries between 1 January 2004 and 31 October 2005 were candidates for the study. Physicians attending the infants in the delivery rooms were asked to save umbilical blood samples for determination of IL-6 and NT-proBNP levels.

During the study period umbilical cord blood samples were obtained from placentas of 100 infants. Excluded from the analyses were 11 infants (including one set of quintuplets and one of quadruplets) because of statistical considerations, and 2 cases where the amount of collected blood was insufficient. Thus, 89 infants with IL-6 levels were available for appraisal; in 66 of them NT-proBNP values were also measured. NT-proBNP was not determined in 23 infants because of insufficient blood volume.

Interleukin-6 levels were measured by a solid-phase enzyme-linked immunosorbent assay [17]; a high sensitive immunoassay kit (Quantikine HS R&D system, Minneapolis, MN 55413, USA) was used. This kit includes an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product. The secondary enzyme system consists of alcohol dehydrogenase and diaphorase (amplifier). NT proBNP was measured with an electrochemiluminescence immunoassay (Elecsys 1010/2010, Roche, Switzerland). The assay is unaffected by icterus, hemolysis or lipidemia.

Demographic, obstetric, perinatal and neonatal data were extracted from the hospitalization files. Most data were already routinely collected at discharge or demise of infants for submission to the Israeli Neonatal Network. The factors considered were maternal age and origin, number of births, type of conception (natural, hormonal or in vitro fertilization), type of delivery (vaginal or cesarean section), single or multiple gestation, pregnancy complications (placental abruption, toxemia, premature rupture of membranes, clinical chorioamnionitis, and premature contractions), steroid treatment received prior to delivery (partial or complete course), gestational age by weeks calculated from the last menstrual period and confirmed by physical examination, birth weight in grams, Apgar scores at 1 and 5 minutes, weight appropriate or small for gestational age, hypotension determined by an initial mean blood pressure (mmHg) lower than gestational age by weeks or by the need for early ionotropic support, blood acidity determined by the worst base deficit measured within the first 12 hours, presence of respiratory distress syndrome, patent ductus arteriosus confirmed by echocardiography, bronchpulmonary dysplasia defined by the requirement of oxygen or mechanical ventilation at a corrected gestational age of 36 weeks, necrotizing enterocolitis (grades 2 or 3), intraventricular hemorrhage (mild: grades I and II, severe: grades III and IV), periventricular leukomalacia, retinopathy of prematurity (grades 2 or 3), surfactant treatment and laser therapy, number of days on mechanical ventilation/oxygen, and days of hospitalization.

Cranial ultrasounds were routinely done within 72 hours of birth and then repeated routinely between 7 and 10 days and at one month. Intraventricular hemorrhage was defined and graded by the criteria of Papille [18]. Periventricular leukomalacia was defined by the presence of echolucent areas around the lateral ventricles. Respiratory distress syndrome was defined by the need for mechanical ventilation by 12 hours of age in an infant with pulmonary radiologic appearance of diffuse granularity.

Eight infants (most on the first day of life) were transferred to other units following delivery because of shortage of room in the neonatal intensive care unit, and eight infants succumbed during hospitalization. Therefore, fewer analyses were performed for postnatal variables than for antenatal ones [Table 1].

The study was approved by the institutional committee responsible for human experimentation. Patient anonymity was assured.

STATISTICAL ANALYSIS

The study group comprised a clustered sample of 57 single babies, 13 pairs of twins, 2 triplets, 1 set of quadruplets and 1 of quintuplets. Evaluation of the intracluster correlation was not possible in the two single clusters of quadruplets and quintuplets and they were therefore excluded from the data analysis. Examining the association of IL-6 with gestational age and with birth weight gave identical results when the multiple births (clusters) effect using the software MLWin for multilevel modeling was considered and when it was not. All subsequent analyses were performed with SPSS V14. Decrease in the percentage of high IL-6 with the variable gestational age was verified by the linear-by-linear-association test.

Fisher's exact test was used to test the association of high IL-6 with dichotomies (antenatal steroid administration, first delivery vs. a higher birth order, pregnancy complications, maternal age or origin, multiple gestation, and being small for gestational age). NT-proBNP values showed a marked asymmetry. Accordingly, the non-parametric Kruskal-Wallis and Mann-Whitney tests were applied. The *t*-test for comparing the NT-proBNP level between cases with high IL-6 was preceded by Levene's test of homogeneity of variances to determine the appropriate number of degrees of freedom.

In our analyses of IL-6, a cutoff of 11 pg/ml was used to distinguish between low and high levels, and the percentage of infants with high levels was compared with the percentage of infants with low levels. In the case of NT-proBNP, mean values were compared.

RESULTS

Cord blood IL-6 levels were measured in 89 infants with a mean ± SD birth weight of 1167.9 ± 313.9 g (range 445–1920 g) and gestational age 28.3 ± 2.1 weeks (range 24–31 weeks). NT-pro BNP levels were also available in 66 of the infants; their mean birth weight was 1170.6 ± 318.4 g (range 445–1800) and gestational age 28.5 ± 1.9 weeks (range 25–31).

IL-6 LEVELS AND PERINATAL VARIABLES [Tables 1 and 2]

IL-6 was high (> 11 pg/ml) in 69% of infants with gestational age < 28 weeks. Increasing gestational age was associated with fewer infants with high levels ($P = 0.02$). Antenatal steroid administration (partial or complete course) was associated with fewer infants with high IL-6 levels ($P = 0.03$). Fewer first-delivery infants had raised IL-6 levels than those of a

Table 1. Antenatal variables: cord blood IL-6 values and levels of NT-proBNP (mean ± SEM)

	IL-6 (pg/ml)			NT-proBNP (ng/L)		
	N	% infants with IL-6› 11	*P* value	N	Mean ± SEM	*P* value
Gestational age						
24–27 wk	29	69.0	0.02	21	3141 ± 550	0.95
28–29 wk	28	55.6		20	3125 ± 678	
30–31 wk	32	34.4		25	4066 ± 1057	
Toxemia						
Yes	10	40.0	0.51	8	3011 ± 1294	0.47
No	79	54.4		58	3552 ± 519	
Premature rupture of membranes						
Yes	33	57.6	0.52	24	2581 ± 382	0.47
No	56	50.0		42	4004 ± 712	
Chorioamnionitis						
Yes	15	13.3	0.15	11	4233 ± 1257	0.06
No	62	50.0		46	3346 ± 602	
Antenatal steroids						
Yes	64	45.3	0.03	50	3608 ± 582	0.90
No	25	72.0		16	3107 ± 792	
Parity						
= 1	35	25.7	0.001	29	2385 ± 251	0.51
› 1	54	70.3		37	4349 ± 809	
Origin						
Jewish	63	49.2	0.35	48	3715 ± 611	0.60
Arab	26	61.5		18	2878 ± 658	
Small for gestational age						
Yes	6	50.0	1.00	4	1561 ± 473	0.28
No	82	53.7		61	3638 ± 513	

Table 2. Postnatal variables: cord blood IL-6 values and levels of NT-proBNP (mean ± SEM)

	IL-6 (pg/ml)			NT-proBNP (ng/L)		
	N	% infants with IL-6 › 11	*P* value	N	Mean ± SEM	*P* value
Apgar 1 min						
‹ 4	6	83.3	0.03	4	4635 ± 3006	0.01
4–7	40	62.5		27	4083 ± 970	
› 7	41	39.0		33	2962 ± 419	
Apgar 5 min						
4–7	13	61.5	0.56	6	7736 ± 3279	0.29
› 7	74	51.4		58	3105 ± 404	
Hypotension						
Yes	31	51.6	1.00	24	4533 ± 1069	0.38
No	47	53.2		35	3047 ± 485	
Respiratory distress syndrome						
Yes	41	53.6	1.00	29	4135 ± 851	0.24
No	38	55.3		28	3128 ± 673	
Bronchopulmonary dysplasis						
Yes	7	42.8	0.70	5	1273 ± 497	0.09
No	63	54.0		45	3355 ± 496	
Mechanical ventilation						
Yes	53	50.9	0.48	37	4439 ± 791	0.09
No	25	60.0		19	2239 ± 351	
Patent ductus arteriosus						
Yes	28	60.7	0.48	22	4732 ± 1110	0.32
No	51	51.0		35	2954 ± 528	
Severe intraventricular hemorrhage						
Yes	16	68.7	0.27	10	3704 ± 1162	0.45
No	62	51.6		46	3687 ± 627	
Necrotizing enterocolitis						
Yes	6	0	0.01	5	3167 ± 1157	0.92
No	71	59.1		50	3776 ± 608	
Periventricular leukomalacia						
Yes	5	40.0	0.65	2	6097 ± 5424	1.00
No	72	55.5		53	3631 ± 559	
Retinopathy of prematurity						
Yes	28	64.3	0,22	17	2822 ± 577	0.93
No	42	47.6		32	3390 ± 644	
Mortality						
Yes	8	75.0	0,28	6	4895 ± 1975	0.36
No	70	52.9		50	3147 ± 457	

higher birth order ($P = 0.001$). Raised levels were also significantly associated with more infants having lower 1 minute Apgar scores ($P = 0.03$).

There were no statistically significant associations with pregnancy complications, maternal age or origin, multiple gestation, and being small for gestational age. There was no association between high levels and early hypotension ($P = 1.00$) and no correlation was found with initial blood acidity ($R = 0.024$, $P = 0.87$). There were no statistically significant differences between groups of infants with or without elevated IL-6 levels with regard to respiratory distress syndrome, bronchpulmonary dysplasia, patent ductus arteriosus, intraventricular hemorrhage, periventricular leukomalacia or retinopathy of prematurity. However, none of the infants who developed grade 2-3 necrotizing enterocolitis had high IL-6 levels ($P = 0.01$).

Similar to previous publications [1,2], we used a IL-6 cutoff of 11 pg/ml in our analyses. We also examined the relationships with perinatal variables, comparing mean values, but no further associations were noted (data not shown).

NT-ProBNP LEVELS AND PERINATAL VARIABLES [Tables 1 and 2]

There were no statistically significant associations between NT-proBNP cord blood level and prenatal variables, such as maternal age and origin, multiple gestation, birth order, steroid administration prior to delivery, gestational age, being small for gestational age or pregnancy complications; although high levels were associated with clinical chorioamnionitis, at borderline statistical significance ($P = 0.06$).

Statistically significant higher NT-proBNP cord blood levels were found in infants with low Apgar scores at 1 minute but not at 5 minutes. The peptide levels tended to be higher in those requiring mechanical ventilation and lower in infants who developed bronchopulmonary dysplasia. The differences, however, were not statistically significant ($P = 0.09$ and 0.09, respectively). There was no association between the levels and other postnatal variables including respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis, periventricular leukomalacia, retinopathy of prematurity, intraventricular hemorrhage, number of ventilation days, and mortality.

RELATION BETWEEN IL-6 AND NT-ProBNP

Linear regression showed no correlation between cord blood IL-6 and NT-proBNP levels ($R^2 < 0.0001$, $P = 0.96$). Of the 66 infants with measured NT-proBNP values, 30 had high IL-6 levels (> 11 pg/ml). There was no difference between NT-proBNP levels in infants with or without elevated IL-6 levels (mean ± SEM 3232 ± 464 vs. 3698 ± 498 ng/L, respectively, $P = 0.63$).

Controlling for gestational age, a weak positive correlation, not statistically significant however, was seen between IL-6 and NT-proBNP levels among infants of 24–27 weeks ($R^2 = 0.151$, $P = 0.08$), but not among the more mature groups ($R^2 = 0.001$, $P = 0.88$ for the 28–29 weeks group, and $R^2 = 0.002$, $P = 0.83$ for the 30–31 weeks group).

DISCUSSION

The correlation of more infants with raised IL-6 levels with decreasing gestational age found in our cohort was reported previously [19] and is in line with the higher prevalence of clinical or subclinical intrauterine infection at an earlier gestation [19]. It has been shown that corticosteroids may alter the ratio of proinflammatory to anti-inflammatory cytokines, resulting in the reduction of the proinflammatory effect [20]. Our finding that most infants who were exposed to antenatal steroids had low IL-6 cord blood levels appears to support these data.

Fewer firstborn infants had elevated cord blood IL-6 levels than infants of higher delivery order. Several studies found a higher rate of intrauterine infections in term nulliparous mothers who had a prolonged second stage of labor and underwent repeat interventions [21]. Others, in line with our results, found evidence for a higher incidence of chorioamnionitis associated with increasing preterm parity [22]. Whether preterm first deliveries are indeed more infrequently associated with intrauterine infection/inflammation should be addressed in future studies.

An association between elevated IL-6 cord blood levels and subsequent neurologic and pulmonary complications in premature infants has been reported by several authors [3-6] but not by others [23,24]. It has been suggested that the varying association of chorioamnionitis and cord blood cytokine concentrations with outcome may be due to differences in population characteristics and treatment practices [24].

More infants with low 1 minute Apgar scores had elevated IL-6 values, suggesting an association with cardiac compromise, but the differences in respiratory distress syndrome, patent ductus arteriosus, bronchopulmonary dysplasia, intraventricular hemorrhage or periventricular leukomalacia were not statistically significant in our study. Regarding necrotizing enterocolitis however, none of the six patients with the disease had high cord blood levels.

High BNP levels were documented in cord blood of infants following fetal distress [15] and fetal heart rate abnormalities [25], suggesting some compromise of cardiac function. The higher levels found in our infants with low 1 minute Apgar scores and the tendency for increased need of mechanical ventilation support the above findings. Since BPD is associated with mechanical ventilation, the low BNP levels found in patients with BPD are intriguing. However, only a minority of the ventilated infants developed BPD (data not shown), and a heterogeneity between these with and without BPD with regard to cardiac function seems plausible. No significant association was demonstrated between NT-proBNP levels and early hypotension, however.

We suggest that higher cord blood NT-proBNP levels may better reflect antenatal adversity, whereas changes during and after delivery may manifest later. The link between high levels and clinical chorioamnionitis, though of borderline statistical significance, may support this notion. Associations with other perinatal variables were not observed in our study.

No correlation was found between IL-6 and NT-proBNP cord blood levels in the analysis of our 66 paired samples. After controlling for gestational age, a weak positive correlation, short of statistical significance however, was demonstrated between Il-6 and NT-proBNP levels in infants of 24–27 weeks gestational age but not among the more mature ones. Both variables have been implicated in neonatal cardiac dysfunction but the pathogenic process is inflammatory in the first instance and related to mechanical pressure in the second. Both IL-6 and NT-proBNP values were raised in infants with low 1 minute Apgar scores, which could be related to some degree of cardiac dysfunction.

Apparently a different time table exists for the evolution of the two markers. The cascade of the inflammatory reaction is antenatal in origin, resulting in high cytokine cord blood levels at delivery, whereas NT-proBNP is released by cardiac myocytes mainly in response to cardiac pressure alterations during and following delivery and may peak later. Accordingly, higher NT-proBNP levels were seen during the first days of life compared with cord blood values [13,14]. Therefore, it is conceivable that a stronger correlation than found in our study may exist between IL-6 cord blood values obtained at delivery and NT-proBNP values sampled at a later stage. Future sequential measurements of the two agents after delivery may better clarify the issue.

In conclusion, we found that high IL-6 and NT-proBNP levels were associated with low 1 minute Apgar scores, whereas a weak positive correlation between the two markers was seen only among the less mature infants. High IL-6 values were more prevalent in early prematurity and without exposure to antenatal steroids, but no significant associations were observed between IL-6 or NT-proBNP cord blood levels and most of the outcome variables. Our results, therefore, do not support utilization of these cord blood levels as the sole tool to predict neonatal outcome.

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Hand-Assisted Laparoscopic Surgery for Liver Tumors

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ABSTRACT: **Background:** The surgical treatment for liver tumors, whether metastatic or hepatic in origin, traditionally used the open approach through large incisions. In recent years the laparoscopic approach became popular but few centers use this method routinely.

Objectives: To assess the results of our initial experience with liver resection using the laparoscopic approach, in terms of patient safety and oncologic surgical outcome.

Methods: Between August 2007 and April 2008 we performed 10 liver resections in 9 patients using the hand-assisted laparoscopic surgery technique.

Results: The main indication for surgery was metastatic colorectal carcinoma in seven patients and hepatocellular carcinoma in two. The mean age was 67 ± 11 years. The tumor was solitary in seven patients. Five patients had neoadjuvant chemotherapy. Altogether, 12 lesions with an average size of 17 ± 9 mm were resected. The mean operative time was 180 ± 52 minutes. Average postoperative stay was 6.5 ± 3.5 days. There was no perioperative mortality. There was one conversion to open surgery due to bleeding from the left hepatic vein. No major perioperative complications were encountered. All resected margins were free of malignancy.

Conclusions: Liver resection using HALS is safe and feasible and should be considered in selected patients.

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KEY WORDS: liver resection, hand-assisted laparoscopic surgery (HALS), metastases from colorectal cancer, hepatocellular carcinoma

Over the past three decades liver surgery has undergone significant advances as a result of improvements in anesthesia and surgical techniques, increased knowledge of the surgical anatomy of the liver, and better understanding of underlying liver disease. These developments have led to more aggressive surgery and to a great reduction in morbidity and mortality. In parallel, laparoscopic surgery was further developed, providing several advantages over surgical interventions, such as reduced postoperative pain and analgesia requirements, shorter hospital stay, lower morbidity, and improved cosmetic results [1].

The liver is a suitable organ for laparoscopic resection because of its deep location and the fact that there is no need for reconstruction after hepatectomy. Laparoscopic liver surgery has been adopted with gradually increasing frequency but has not entered routine use because of the inability to assess safe margins of resection owing to loss of tactile sense and difficulty with safe parenchymal transaction. Recent technological advances have made laparoscopic liver resection possible. These include laparoscopic ultrasound, introduction of the stapling technique in liver surgery [2], development of parenchymal transaction tools such as the LigaSure and the harmonic scalpel [3], and the addition of the hand assistance port [4].

This study presents our initial experience with hand-assisted laparoscopic liver resection for malignant tumors, with emphasis on patient safety and short-term outcomes.

PATIENTS AND METHODS

Between August 2007 and April 2008 we performed 10 laparoscopic hepatic resections in 9 patients for malignant liver disease using the HALS technique. Patients who were potential candidates for hepatic resection in our department were evaluated at a weekly multidisciplinary round.

Indications for laparoscopic resection were similar to those in traditional surgery. Resection of hepatocellular carcinoma was considered when there were no more than two nodules in well-compensated chronic liver disease (Child-Pugh class A), without signs of portal hypertension and without tumor vascular invasion. Hepatic resection for liver metastases from colorectal carcinoma was considered only in the absence of extrahepatic disease.

The preoperative workup included blood examinations, tumor markers, imaging modalities (computed tomography, positron emission-CT, magnetic resonance imaging, ultrasound), and characteristics of the specific tumor (number, location, size and relation to intrahepatic vascular or biliary structures). Exclusion criteria for laparoscopic resection included gallbladder carcinoma, portal hypertension or decompensated chronic liver disease, and cardiac or respiratory failure.

HALS = hand-assisted laparoscopic surgery

The patients underwent standard evaluation for major surgery by an anesthesiologist. All the patients were informed of the nature and morbidity of the procedure and gave informed consent.

HALS TECHNIQUE

Liver resections were defined according to the International Hepato-Pancreato-Biliary Association terminology derived from the Couinaud classification. Deep segments included segments I, IVa, VII and VIII, while superficial segments included segments II, III, IVb, V and VI. Resection was considered "anatomic" when at least one entire segment was removed; all other resections were defined as non-anatomic or wedge resections. Left lateral lobectomy was defined as resection of segments II and III.

All the operations are performed with the patient under general anesthesia. The patient is placed supine in the "French" position with the primary surgeon positioned between the patient's spread legs and one assistant on the left. The procedure begins with a right subcostal incision far enough from the costal margin. The exact site of the incision is chosen to allow control of both right and left lobes by the surgeon's left hand. The surgeon dissects the adhesions and then inserts the hand port (LapDisc®, Ethicon, Cincinnati, OH, USA). Through the hand port, now with the addition of the surgeon's left hand, a trocar is inserted into the abdominal cavity, the pneumoperitoneum is produced with CO_2 at a pressure of 12–15 mmHg, and visual exploration of the liver and the abdominal cavity is conducted with a 30 degree laparoscope. A 12 mm infraumbilical trocar is then inserted into an adhesion-free area, followed by two to three additional working trocars placed according to the location of the liver lesions, usually along a semicircular line with the concavity facing the right subcostal margin. We use balloon port trocars (Covidien®, Norwalk, CT, USA), which are advantageous since they provide a mechanism to create upward traction on the abdominal wall, thereby enhancing working space for appropriate articulation of the endovascular staplers. The abdominal cavity is explored manually and intraabdominal sonography of the liver is performed. Ultrasound is used to confirm the extension of the tumor, determine the number of lesions, identify potentially hazardous intrahepatic vascular or biliary structures, and demarcate surgical tumor resection margins. The hepatic pedicle is never encircled for the Pringle maneuver.

In our patient the liver parenchyma transaction was performed using the LigaSure™ (LigaSure 5 mm; Valleylab, Boulder, CO, USA). If needed, clips or endoGIA™ staplers (vascular cartridge) (EndoGIA, Covidien, Norwalk, CT, USA) were used for large parenchymal vessels or biliary structure.

For left lateral lobectomy, the round ligament was divided close to the abdominal wall with a harmonic scalpel (Ultracision™, Ethicon Endosurgery, Cincinnati, OH, USA). The falciform ligament was divided from the anterior abdominal wall towards the inferior vena cava, and the left triangle ligament was divided to free the left lobe. The lateral wall of the left hepatic vein was exposed and care was taken to avoid injury to the left hepatic and phrenic veins. The posterior surface of segment II-III was exposed and the lesser omentum was checked for the presence of a left hepatic artery. The lesser omentum was divided by 5 mm LigaSure. When a bridge of liver parenchyma covered the round ligament it was divided with the 5 mm LigaSure. The liver was transected on a line just left of the falciform ligament using 5 mm LigaSure until the portal pedicle of segments II-III were exposed. The portal pedicle of segments II-III was divided with EndoGIA staplers (vascular cartridge) applied two or three times. The transaction was continued until the left hepatic vein was reached, after which it was stapled with a small amount of surrounding liver tissue. The specimen was extracted through the LapDisc. The argon beam was used for hemostasis. A single, flat Jackson-Pratt drain was placed in the posterior aspect of the resection bed through a port site. All specimens were sent fresh for pathologic examination to measure the surgical margins.

RESULTS

From August 2007 to April 2008, nine patients with malignant liver tumors were selected for the HALS technique. The main indication for operation was metastatic colorectal carcinoma in seven patients and hepatocellular carcinoma in two. Table 1 presents the indication for resection, medical history and preoperative neoadjuvant chemotherapy. The mean age of the patients – 3 (33%) women and 6 (67%) men – was 67 ± 11 years (range 50–82 years). The tumor was solitary in

Table 1. Characteristics of patients undergoing HALS liver resection

Patient	Age (yrs)	Gender	Diagnosis	Medical history	Location (segment)	Neo-adj treatment
1	70	F	LMCRC	HTN	II-III	Yes
2	51	M	LMCRC		V	Yes
3	64	F	HCC	HTN, COPD	VIII	No
4	82	M	LMCRC	HTN, IHD, CVA	VI	No
5	62	M	LMCRC	Epilepsy	II-III	No
6	77	M	LMCRC	IHD	II-III	Yes
7	71	F	LMCRC	HTN	II-III	Yes
8	77	M	HCC	HTN, DM, IHD, COPD	V	No
9	52	M	LMCRC		II-III, VIII	Yes

LMCRC = liver metastases from colorectal carcinoma, HTN = hypertension, HCC = hepatocellular carcinoma, COPD = chronic obstructive pulmonary disease, IHD = ischemic heart disease, DM = diabetes mellitus

Table 2. Surgical procedure and perioperative data

Patient	Procedure	Lesions	Size (mm)	Margins (mm)	Operative time (min)	Morbidity	Stay (days)
1	LLL+AR	1	35	10	197	Yes	7
2	Seg V	1	25	10	153	No	3
3	Seg VIII	1	30	11	252	No	5
4	Seg VI	2	15, 6	7, 8	165	No	4
5	LLL	1	40	10	135	Yes	8
6	LLL	1	14	14	196	No	5
7	LLL	1	20	15	88	No	5
8	Seg V	1	30	1	248	Yes	15
9	LLL+Seg VIII	3	11, 13, 8	12, 2	180	Yes	7

LLL = left lateral lobectomy, AR = anterior resection, Seg = segment

seven patients, while one patient presented with two nodules and another patient with three nodules. Five patients with colorectal liver metastases had neoadjuvant chemotherapy, usually four to six cycles of Folfox and Avastin® (Roche, Israel). One patient had combined surgery, where anterior resection and left lateral lobectomy of the liver were completed using HALS.

OPERATIVE RESULTS

Altogether, 12 lesions with an average size of 17 ± 9 mm were resected. Ten liver resections were performed; types and details of the liver resection are shown in Table 2. When we first began using this approach we had one conversion (11%) due to mild bleeding from the left hepatic vein during left lateral lobectomy. This patient required one transfusion of packed blood cells; he was included in our statistical analysis. There was no perioperative mortality. The mean operative time was 180 ± 52 minutes. Average postoperative stay was 6.5 ± 3.5 days. Four patients had a postoperative complication: wound infection, pneumonia, urinary tract infection, and an abscess that was drained percutaneously, respectively.

PATHOLOGIC RESULTS

Ten metastatic colorectal lesions were resected from 7 patients. One colorectal metastatic mass after chemotherapy was resected but revealed no malignancy in the final pathology. All lesions were resected with tumor-free margins of 9 ± 5 mm.

DISCUSSION

Since the introduction of laparoscopic cholecystectomy in 1987, laparascopic techniques have been applied for solid organ surgery. The first non-anatomic liver resection for focal nodular hyperplasia was described by Gagner et al. [5] and the first left lateral lobectomy by Azagra et al. [6]. Despite the widening of indications and improved surgical equipment for hepatic surgery, the endoscopic approach remains underutilized for liver surgery as compared to other surgical modalities. Due to specific difficulties associated with this procedure it is not commonly used by hepatobiliary surgeons worldwide. Loss of manual palpation of the liver during laparoscopy may compromise the oncologic resection. A long learning curve is required to reduce the risk for intraoperative complications such as uncontrolled bleeding biliary injury and laparoscopy-specific complications.

The current literature contains accounts of both pure laparoscopic resections and hand-assisted laparoscopic resections, with firm support for both [7-13]. Our center uses the hand-assisted approach for reasons of patient safety and safe practice of oncologic surgery. Left lateral lobectomy is the most frequently performed laparoscopic procedure due to excellent exposure of the whole operation field and safe vascular control [8,11,14,15]. In most of our patients (55%) a left lateral lobectomy was performed, using a simple stapling technique without inflow or outflow control. Resection of other segments can be more problematic; lesions in easily accessible lower liver segments (IVb, V, and VI) with minimal parenchymal transaction are still considered the best indication for a laparoscopic approach [1]. Lesions of the posterior and superior liver segments (IVa, VII, and VIII) are technically demanding, especially in terms of choosing the right transaction plane and controlling bleeding [7,8]. In our initial experience we performed resections for tumors that were confined to segments II-VI (the laparoscopic segments), which were relatively easy to manipulate, were far from large hepatic veins and porta hepatis, and bleeding could be visualized and controlled laparoscopically with LigaSure, stitches, endoGIA or clips. We performed non-anatomic resection of segment VIII and resection of deep segment V; the operative time in these cases was significantly longer than in resection of the other laparoscopic segments. Intraoperative ultrasound is mandatory; it allows correct staging of the tumor, precise evaluation of its extension and its relationship with major surrounding structures, and oncologic free margins.

The size of the tumor is important when selecting patients for laparoscopic resection, in which an acceptable diameter for nodular and pedunculated tumor should be smaller than 40 mm and 60 mm respectively [1]. In our series, the mean tumor size was 17 mm (range 6–40 mm).

The main concern during liver resection is bleeding. Parenchymal bleeding is usually limited and can be controlled either with the full laparoscopic approach or HALS. Hepatic veins or arterial bleeding put the patient at immediate risk. This major bleeding can be efficiently controlled by the surgeon's hand, which is inside the patient's abdominal cavity throughout the HALS procedure. Hemorrhage is the main reason for conversion to open surgery, with rates

ranging from 0% to 55%. In our series one procedure (11%) was converted to open surgery due to bleeding from the left hepatic vein. This occurred at the start of our experience; we prefer to convert to open surgery for patient safety.

There was no perioperative mortality in our experience. In general, acceptable perioperative mortality in liver surgery is ≤ 5%. HALS or full laparoscopy series have demonstrated very low mortality rates (1.2% and 1% in two series and zero in all other reports). These low rates can be explained by patient selection, i.e., patients with tumors located in less risky segments.

All resected tumors in our series were excised with healthy margins. The largest lesion measured 40 mm, but much larger lesions can be resected using HALS since most incisions are 7.5 cm in length. Long-term follow-up is required to ascertain that port site or peritoneal spread did not occur. This was reported in other series of laparoscopic abdominal operations for malignancy of the gastrointestinal tract; the rates of recurrence were low and the procedure was considered practically safe.

In our series the average postoperative in-hospital stay was 6.5 days, which reflects a prompt recovery due to small incisions and early mobilization, which are seen less when laparotomy is used.

In conclusion, we found that the HALS technique approach for the treatment of solid tumors in the liver for highly selected patients is safe, feasible and easily tolerated by patients; it has a good cosmetic result and does not compromise the oncologic and pathologic standards of care. Long-term follow-up is still needed to establish the surgical superiority of the HALS technique over traditional laparotomy in terms of a disease-free outcome, incisional complications and patient satisfaction.

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Capsule

Atherosclerosis inhibiting leukocytosis

Leukocytosis – an elevated white blood cell count – contributes by unknown mechanisms to the pathogenesis of atherosclerosis and associated coronary heart disease. Yvan-Charvet et al. show that the adenosine triphosphate-binding cassette transporters ABCA1 and ABCG1 are critical suppressors of atherosclerosis-associated leukocytosis. Mice deficient in both transporters in blood-producing hematopoietic cells possess increased levels of hematopoietic stem and multipotential progenitor cells and accelerated atherosclerosis. ABCA1 and ABGA1 protect against atherosclerosis by promoting cholesterol efflux from cholesterol-laden macrophage foam cells to lipid-poor high density lipoprotein (HDL) and apolipoprotein A-1. The leukocytosis and atherosclerosis in ABCA1- and ABG1-deficient mice were reversed in the presence of high amounts of HDL. Thus, signaling already known to inhibit atherosclerosis by reducing cholesterol in atherosclerotic plaques also reduces atherosclerosis-associated leukocytosis.

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Eitan Israeli

"Everyone is kneaded out of the same dough but not baked in the same oven"

Yiddish proverb

Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infections in Israel*

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ABSTRACT: **Background:** Community-associated methicillin-resistant *Staphylococcus aureus* infections are increasingly being documented worldwide. In Israel, however, CA-MRSA infections have not yet been reported, so awareness among physicians may be low.

Objective: To alert physicians to the possibility of CA-MRSA infection, which necessitates a distinct therapeutic approach.

Methods: We present three children with soft tissue infections caused by CA-MRSA who were treated in our medical center from January to March 2009.

Results: In all three cases CA-MRSA was identified as the causative pathogen after surgical or spontaneous drainage. On susceptibility testing, the organisms were resistant to beta-lactam antibiotics but susceptible to clindamycin, rifampicin and trimethoprim-sulfamethoxazole.

Conclusions: Physicians should maintain an index of suspicion for CA-MRSA infections. The antibiotic-resistance profile of *S. aureus* should be watched carefully, and in particular, cultures should be obtained whenever soft tissue infections fail to respond to conventional treatment.

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KEY WORDS: *Staphylococcus aureus*, soft tissue infection, antibiotic resistance, vancomycin, clindamycin

Methicillin-resistant *Staphylococcus aureus* is a well-established nosocomially acquired pathogen [1]. The more recent community-associated MRSA infection, first reported in the United States in 1981 [2], has shown an alarming worldwide spread. Its high virulence and complication rates, together with the wide spread, have led to changes in the therapeutic approach to presumed staphylococcal infections acquired in the community [3].

We are not aware of previous published reports of CA-MRSA infections in Israel. However, nasal carriage of MRSA in the community [4] and MRSA bacteremia in a neonatal intensive care unit with antibiotic susceptibility pattern similar to CA-MRSA have been described in Israel [5]. In this work, we present three children with soft tissue infections caused by CA-MRSA. Our aim was to prompt physicians to be more alert to the possibility of infection with CA-MRSA, which has a distinct antibiotic susceptibility profile and necessitates a distinct approach regarding empiric therapy.

PATIENT DESCRIPTIONS

Three patients with MRSA infections acquired in the community were treated in our medical center from January to March 2009 [Table 1]. All presented with soft tissue infections and were initially treated empirically with a first-generation cephalosporin, which is inappropriate for CA-MRSA. CA-MRSA was identified as the causative pathogen only after surgical or spontaneous drainage. Laboratory analysis showed that the organisms were resistant to beta-lactam agents but susceptible to clindamycin, rifampicin, and trimethoprim-sulfamethoxazole.

PATIENT 1

A 2 year old girl presented to the emergency department with fever and left inguinal swelling and redness. Physical examination revealed an inguinal abscess with spontaneous excretion of pus. The white blood cell count was 16,490 x 10^3/μl with 75.1% neutrophils, and the C-reactive protein level was 7.4 mg/dl. After obtaining pus for culture, we admitted the patient to the pediatric ward where she was treated intravenously with a first-generation cephalosporin (cefazolin). The fever resolved but the local findings persisted. The treatment was therefore empirically changed to intravenous amoxicillin-clavulanate and clindamycin. On the third day of hospitalization we received the laboratory report of a positive culture for MRSA. The child was later transferred to the surgical ward to drain the abscess; she was later discharged and prescribed oral trimethoprim-sulfamethoxazole for one week.

***After this paper was submitted the following article was published: Glikman D. Community-associated methicillin-resistant *Staphylococcus aureus* infections among children in the Western Galilee region: the beginning of an epidemic? *Harefuah* 2009; 148(11): 761-5, 793, 794 (Hebrew).**

CA-MRSA = community-associated methicillin-resistant *Staphylococcus aureus*

Table 1. Background, clinical and laboratory characteristics of three patients with CA-MRSA infection

Patient #	Age	Gender	Site of infection	Initial treatment	Surgical drainage	Time to diagnosis of MRSA	Antibiotic susceptibilities	Outcome
1	2 yrs 3 mos	F	Left groin	Cefazolin	Yes	3 days after admission	Clindamycin Rifampicin Trimethoprim-sulfamethoxazole Vancomycin Chloramphenicol Gentamicin Minocycline	Recovery
2	4 yrs 2 mos	M	Left temporal region	Cefazolin	Yes	13 days after admission		Recovery
3	14 yrs 5 mos	M	Left buccal region	Cephalexin	Yes	5 days after presentation		Recovery

PATIENT 2

A 4 year old boy presented to the emergency department with fever, left facial swelling and trismus of 3 days duration. Two weeks previously he had presented at the emergency department because of pneumonia and was discharged with oral amoxicillin. Physical examination at the latest admission revealed left facial swelling and tenderness and a left reactive submandibular lymph node. The WBC count was 16,070 x 10^3/µl with 38.6% neutrophils, and the CRP level was 1.29 mg/dl. The child was admitted to the pediatric ward and treated with intravenous cefazolin. Minor systemic and local improvements were noted, but significant swelling and tenderness remained. Magnetic resonance imaging was subsequently performed, demonstrating an abscess within the temporalis muscle, probably due to an infected congenital cyst. The abscess was surgically drained and culture of the aspirate yielded MRSA. After susceptibility testing, the antibiotic treatment was changed to IV vancomycin and oral trimethoprim-sulfamethoxazole. The clinical signs and symptoms resolved, and the child was discharged with a recommendation for ambulatory follow-up, including MRI.

PATIENT 3

A 14 year old boy presented to the emergency department with a 1 day history of fever and left facial swelling following a local acne lesion. He was discharged with an oral first-generation cephalosporin (cephalexin). Two days later he returned because the fever persisted and the swelling worsened. Physical examination revealed local warmth, redness, swelling and tenderness of the left cheek. The WBC count was 14,820 x 10^3/µl with 64% neutrophils and the CRP level was 9.12 mg/dl. The patient was admitted to the pediatric ward and treated intravenously with a first-generation cephalosporin (cefazolin). Pus was drained from the swelling and sent for culture. The patient recovered and was discharged home with instructions for another 5 days treatment with cephalexin. The culture grew MRSA, but since the patient had recovered there was no clinical need for further antimicrobial treatment.

WBC = white blood cell
CRP = C-reactive protein

DISCUSSION

Soon after the introduction of penicillin in 1941, resistant strains of *S. aureus* emerged, first in hospital settings and then disseminated in the community [6]. Today, most *S. aureus* strains are resistant to penicillin. *S. aureus* resistance to other penicillins and cephalosporins has been undergoing a similar process as more and more strains have acquired the *mecA* gene that encodes penicillin-binding protein 2A [7]. Initially, for a relatively long period, MRSA infections were limited to hospitals, mainly intensive care units [8]. In recent years, however, they have been increasingly reported in the community in many locations worldwide [9]. In certain areas, the majority of community-associated *S. aureus* infections are now methicillin resistant [10]. Infections caused by CA-MRSA are severe and often complicated and have the potential for a high mortality rate [11,12].

The optimal antimicrobial management of the rapidly emerging CA-MRCA has not been completely elucidated [11-13]. It is obvious that appropriate surgical drainage is of prime importance and in mild cases may be sufficient, as our third case exemplified. Most isolates of CA-MRSA are susceptible to clindamycin, in contrast to nosocomial MRSA. Clindamycin can therefore be used, although clindamycin-resistant CA-MRSA was recently reported [14]. CA-MRSA is usually susceptible to trimethoprim-sulfamethoxazole, which can be used in mild cases, although it has not been approved by the U.S. Food and Drug Administration to treat staphylococcal infections [15]. These strains are always susceptible to vancomycin.

Practice guidelines for the diagnosis and management of skin and soft tissue infections still recommend the empiric use of semisynthetic penicillin, first- or second-generation cephalosporins, macrolides, or trimethoprim-sulfamethoxazole, with reevaluation within 48 hours pending culture results [13]. Our first patient received amoxicillin-clavulanate and clindamycin, to which the isolated CA-MRSA was susceptible, and was discharged on oral trimethoprim-sulfamethoxazole. In the second patient, vancomycin was started as we had been notified ahead on the growth of MRSA.

Several studies have attempted to elucidate the risk of

CA-MRSA in Israel. Schlesinger et al. [16], in an investigation of the prevalence of MRSA carriage in children in Jerusalem, found that MRSA colonized the nares of 0.6% of healthy children, whereas among chronically institutionalized children the carrier rate was 7.6%. In 2002, an outbreak of MRSA infection was reported in a chronic care institution for mentally retarded adults in Israel [17].

To the best of our knowledge, the present work is the first published description of MRSA infection acquired in the community in Israel, although nasal carriage of MRSA in the community [4,16] and MRSA bacteremia in neonatal intensive care units with antibiotic susceptibility pattern similar to CA-MRSA were described previously in Israel [5]. It is noteworthy that patient 2 had been treated with amoxicillin in the emergency department 2 weeks before he presented with the abscess, probably an infected cyst, caused by MRSA. Given the course of the infection, and considering that he was not treated with anti-staphylococcal penicillin or a cephalosporin, we assumed the soft tissue MRSA infection was acquired in the community. Patient 3 recovered from the infection after surgical drainage without the need for an additional antimicrobial agent, highlighting the major role of surgical drainage in CA-MRSA infections. The antimicrobial susceptibility profile of the CA-MRSA strains was similar in all three cases and was typical of CA-MRSA as opposed to nosocomial MRSA. Molecular studies to look for clonality of these three strains were not performed.

In conclusion, physicians should be alert to the possibility of infection caused by CA-MRSA. The antibiotic susceptibility pattern of staphylococcal infections should be monitored carefully and cultures should be obtained, especially when soft tissue infections fail to respond to conventional antimicrobial treatment.

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"Perfectionism is the enemy of creation, as extreme self-solitude is the enemy of well-being"

John Updike (1932-2009), American novelist, poet, short story writer, art critic, and literary critic. A Pulitzer Prize winner, he is considered one of the great American writers of his time

"I believe in evidence. I believe in observation, measurement, and reasoning, confirmed by independent observers. I'll believe anything, no matter how wild and ridiculous, if there is evidence for it. The wilder and more ridiculous something is, however, the firmer and more solid the evidence will have to be"

Isaac Asimov (1920-1992), Soviet-born American author and professor of biochemistry at Boston University, best known for his works of science fiction and for his popular science books

Mohs Micrographic Surgery: Current Techniques

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KEY WORDS: Mohs micrographic surgery, frozen sections, skin tumors, tissue conservation, histologically clear margins

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For Editorial see page 441

Mohs micrographic surgery is considered the most conservative yet reliable approach to the management of cutaneous malignancies. The concept of MMS is simple, but its technique, which involves a series of suboperations, is complex. Although many refinements have been made to the original Mohs technique, the main objectives are the same. MMS represents a method of treating skin cancers in staged excisions using meticulously mapped-out peripheral sections of margins that completely encompass the neoplasm. The surgeon is the one who examines the tissue specimens, aiming at maximal tissue conservation while assuring histologically clear margins [1]. MMS yields cure rates that exceed those of all other modalities, while allowing for maximal healthy tissue conservation.

Mohs micrographic surgery yields cure rates exceeding those of all other modalities while allowing for maximal conservation of healthy tissue

The technique of MMS has continued to evolve since its inception and is currently the treatment of choice for skin tumors in critical sites, in sites of radiation therapy, large or recurrent tumors, and tumors with aggressive histologic features. We review the commonly used MMS techniques available and the indications for MMS.

HISTORY

In 1941, Frederic Mohs described a new surgical technique for staged removal of skin cancer by in situ fixation of cutaneous tissue [2]. After fixation, Mohs excised the cancer and cut tangential sections including both the epidermis and deep undersurface of the tissue sample for microscopic margin evaluation. The "horizontal" sectioning allowed for complete examination of the peripheral tumor margin. Since then, his technique has continued to evolve. It has become the gold standard and has found a secure niche in the management of cutaneous malignancies.

CUTANEOUS MALIGNANCY

Several modalities are used for the treatment of skin cancers, including curettage and electrodessication, laser, cryotherapy, radiation therapy, topical or intralesional drug therapy, conventional excision, and MMS. Laser, cryotherapy and radiology are destructive procedures that rely on clinical and often visual assessment of the tumor's extent but lack pathologic verification of clear margins. ED&C does not permit margin control, although in skilled hands pathologic and normal tissue can often be differentiated with the curette. Conventional excision, using 3–6 mm of clinically diagnosed tumor-free skin margins, is used for most skin cancers [3]. Conventional excision is usually followed by limited pathologic evaluation of margins [4] [Figure 1A]. In contrast, MMS aims to assess 100% of the peripheral and deep margins of the specimen. This is based on the novel histopathologic technique developed by Mohs. Although conventional treatment modalities generally result in high cure rates for small, well-circumscribed non-melanoma skin cancers, the highest overall cure rate for primary as well as recurrent tumors is achieved with MMS [5,6]. (The 5 year recurrence rate of primary and recurrent basal cell carcinoma treated by surgical excision is 10% and 17% respectively; with MMS the rate is 1% and 6% [6].)

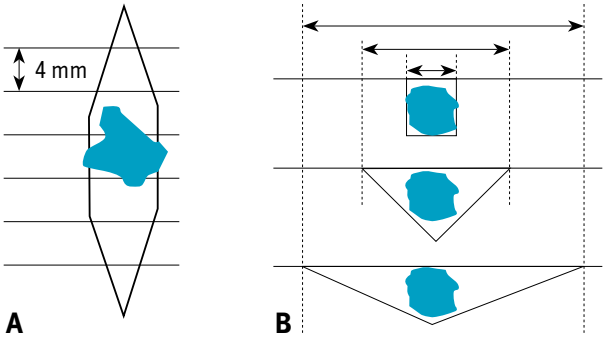
PATIENT EVALUATION

The indications for MMS are well established, especially for non-melanoma skin cancer. Most patients are referred for MMS after a biopsy has been performed and the presence of a carcinoma is confirmed. These are patients with recurrent tumors; tumors in the central face, periorbital,

MMS = Mohs micrographic surgery

ED&C = electrodessication and curettage

Figure 1. [A] Serial transverse cross-sectioning (bread-loafing) technique for elliptical excision specimens. Tissue is sectioned every 4 mm, embedded in paraffin, and cross-sections are cut and stained. This technique can miss portions of tumor at the surgical margins. Tumor is indicated by gray lines, surgical incisions by solid black lines, and horizontal parallel lines signify where transverse sections would be cut. Because the tumor at the incision line is not transected by the cross-sections cut, the tumor would be histologically missed.
[B] Surgical margins required for invasive tumors based on angle of incision. With a vertical incision, an invasive tumor can be excised with minimal lateral margins, resulting in the smallest surgical defect. Excisions with scalpels beveled at 45° and 30° result in progressive increases in the size of the surgical defect necessary to clear the deep portion of the tumor.



nose, lip, and auricle; tumors larger than 2 cm in diameter; tumors with aggressive histology; tumors in the immunosuppressed patient; and irradiated skin [7,8] [Table 1]. The role of MMS in the treatment of other tumors such as melanoma and Merkel cell carcinoma is more controversial and often depends on the surgeon's comfort and preference.

Table 1. Indications for Mohs surgery [7,8]

Recurrent tumors
• Large tumors › 2 cm in diameter
• Tumors that are incompletely excised
• Tumors located in areas where the risk of local recurrence is high (i.e., the central face, auricle, periorbital and periauricular areas)
• Tumors located in areas where tissue conservation and a high cure rate are important
• Tumors with indistinct clinical margins
• Tumors with aggressive histologic subtypes (micronodular, infiltrative and morpheaform basal cell carcinoma, basosquamous carcinoma and poorly differentiated squamous cell carcinoma)
Tumors with evidence of perineural invasion
• Tumors arising in irradiated skin or in chronic scars

SURGICAL TECHNIQUE

The effectiveness of the Mohs technique is dependent on the individual steps that constitute the surgical procedure. These steps include preoperative physical examination, skin tumor extirpation, tissue mapping, histologic processing and microscopic examination. The procedure is repeated until negative margins are confirmed. The postoperative defect is repaired by an optimal reconstructive technique.

MMS is the treatment of choice for skin tumors in critical sites, in sites of radiation therapy, recurrent tumors, and tumors with aggressive histologic features

When initially examining a patient, the clinical margins of the tumor are evaluated and marked with a surgical marking pen. The patient is then asked to verify the location of the tumor with a mirror and confirm the patient's identity. Local anesthesia is obtained with an injection of lidocaine mixed with epinephrine buffered with sodium bicarbonate. A curette may be used, prior to excision, to debulk and delineate possible subclinical tumor spread [9,10].

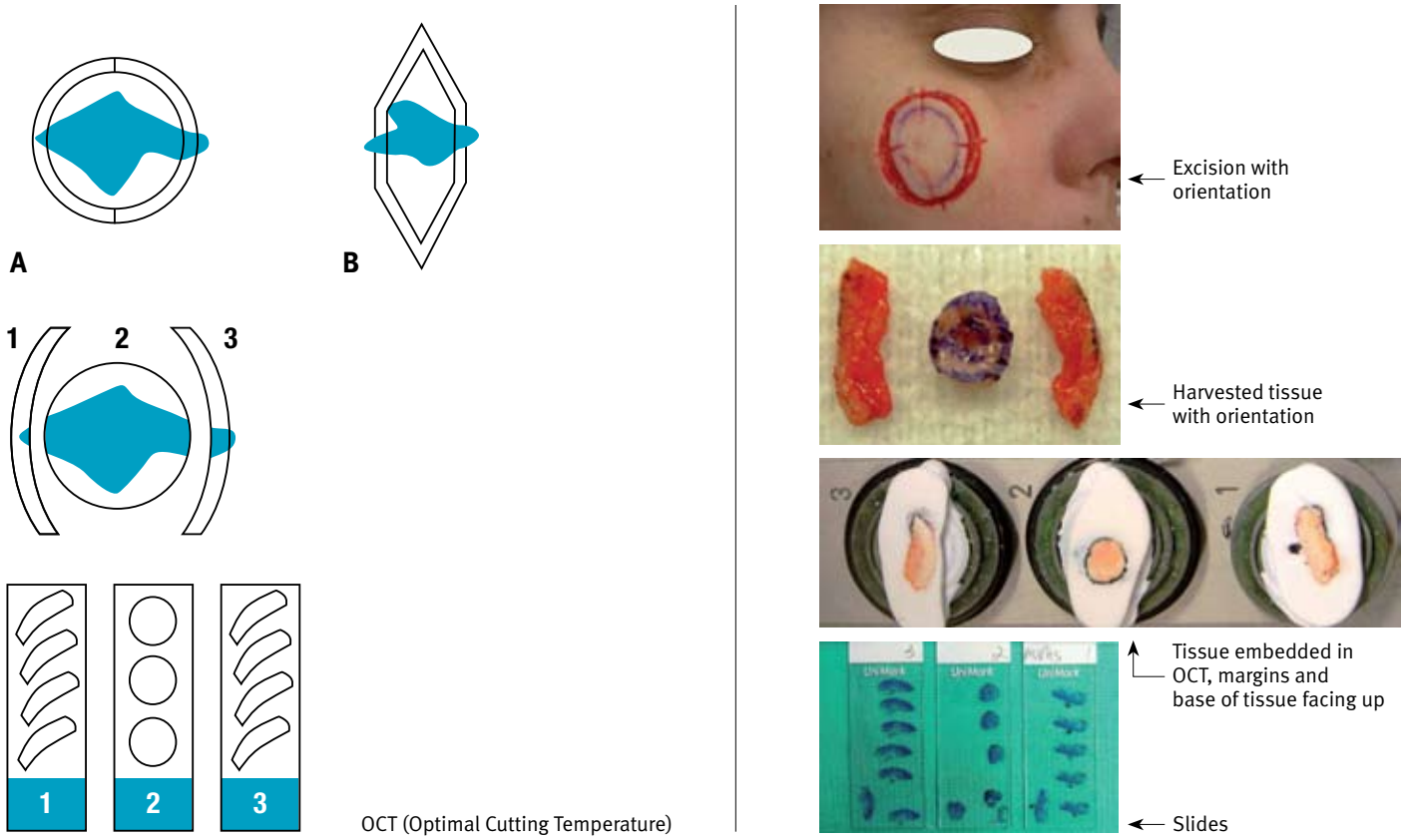
A study evaluating the effectiveness of performing curettage before MMS for previously biopsied non-melanoma skin cancers concluded that although curettage may be helpful in debulking friable skin prior to MMS, it does not reliably delineate the entire extent of a tumor [11]. In addition, preoperative curettage may not reduce the number of stages of MMS. When treating a patient with MMS, one must consider the fact that after a biopsy 24% of non-melanoma skin cancers have no residual component when examined histologically [12]. For such tumors, aggressive curettage can create a larger defect, with no potential improvement in the accuracy of the procedure. Furthermore, this technique is limited in the face of larger tumors that cannot be processed as a single section.

One of the major advantages of MMS is its potential to allow the surgeon maximal normal tissue conservation. With the histologic control the surgeon can be confident that the tumor is no longer present, even when narrow margins are taken.

Classical MMS advocates that the blade be beveled at a 45° angle to the skin surface when excising the tumor margin. This allows the epidermis, dermis and deeper tissue to be cut on the cryostat in a straight line and to be examined in one plane [13]. However, this does not guarantee a complete epidermal edge for histologic evaluation in every case [14]. A more complete epidermal edge can frequently be obtained by using a peripheral 90° vertical incision all around a neoplasm and examining separate horizontal sections of the tumor's base to evaluate the deep margin [1,15] [Figure 2]. Many consider this approach equally effective compared to the oblique 45° sections. Furthermore, the 90° removal approach may ultimately speed up the procedure by obviating the need to incise the margins vertically at 90° and excise the beveled edge to facilitate closure [Figure 1B and 2B].

After tumor excision, the excised tissue must be accurately mapped and marked with ink for proper orientation. Despite a unified concept of margin control for tumor extraction, there are several variations in each step of this technique. A

Figure 2. Combinations of a peripheral 90° vertical incision and horizontal sections of the tumor's base to evaluate histologic margins. The tumor may be excised in a circular **[A]** or elliptical shape **[B]**. Typically, a 1–2 mm margin is initially taken around the tumor for the first stage [10].
The right side of the figure shows a patient with the tumor incised, the tumor specimen divided into three pieces, the tissue pieces embedded in OCT, and the microscopic slides with the stained sections.



survey by Silapunt et al. [16] of 310 Mohs micrographic surgeons in the United States between October and December 2002 showed that most Mohs surgeons mapped their tissue using hand-drawn pictures to orient the specimens. This method is inexpensive, simple and quick, and gives the surgeon artistic freedom to illustrate, on the patient, the size and shape of neoplastic tissue as well as the actual defect. Preprinted maps or sketches of anatomic sites are used by 21% of Mohs micrographic surgeons. It is as simple and rapid as drawing a picture by hand, except that the size and shape of the anatomic regions are fixed. Neither hand-drawn pictures nor preprinted maps can provide an accurate record from which to evaluate the skin in the case of recurrent disease. Digital and Polaroid photographs produce the most accurate representations of the excised tissue, defects and their interrelationship. They may also provide dimensions and archival information for follow-up. Currently, this approach is used by a minority of Mohs surgeons (fewer than 2%), but it may increase with the more widespread use of digital photography and electronic medical records.

MMS is performed by a team of surgeons, nurses and histotechnicians, whose cooperation and communication are crucial for ensuring the efficiency and integrity of the technique

Preparing tissue specimens for processing involves inking, flattening, freezing, cutting and staining before the microscopic examination. The histotechnician plays a crucial role in this process and must consistently orient the tissue so that the correct epidermal surface is sectioned. Mohs surgeons should know how to flatten, freeze, cut and stain tissue in order to efficiently communicate and troubleshoot quality issues with their histotechnician.

The harvested tissue is then embedded in optimal cutting temperature compound and frozen sections are prepared. Flattening the tissue in order to section the complete undersurface and the epidermal margin at the same time is critical for the complete en-face examination of the outer

margin of a tissue specimen. Heat-extractor flattening in the cryostat with or without relaxing tissue cuts or slits is the most common method used in tissue flattening. Relaxing cuts are particularly useful when thick specimens are obtained for processing. Aerosol or liquid nitrogen freezing on a flap glass slide, plastic plate, or X-ray film is another technique commonly used to flatten tissue. More than one method is frequently used for a particular specimen.

The tissue is then sectioned and slides are prepared for histologic evaluation. Before this, however, the tissue sections are stained so that the histologic features can best be appreciated. Hematoxylin and eosin is the most commonly used tissue stain in MMS (82.6%). H&E can be used for all cutaneous neoplasms including squamous cell carcinoma, basal cell carcinoma, melanoma and others. Toluidine blue is an alternative stain that is particularly useful when evaluating basal cell carcinoma. Toluidine blue highlights islands of basal cell carcinoma by metachromatically staining its surrounding mucopolysaccharides a vibrant pink color [17]. The use of both toluidine blue and H&E for different sections of the same specimen has been advocated when the assessment of a tumor is not straightforward.

Epidermal and dermal specimens are most commonly sectioned 5 to 6 mm thick and fatty tissue is cut at 15–25 μm [18]. Sections that are too thick are difficult to evaluate and can lead to inaccurate interpretation, while thinner sections enhance cellular detail. Serial sectioning of the tissue specimens allows one to further differentiate normal adnexal structures from tumor nests. In fact, inadequate sectioning may result in falsely positive margins. Although more processing time is required for serial sectioning, it may reduce the number of equivocal readings at tumor margins.

For basal cell carcinoma < than 10 mm in diameter, conventional excision margins of 3 mm will have a cure rate of 85%. A 4 mm margin will produce about a 95% cure rate [19]; in larger, sclerosing or recurrent basal cell carcinoma, a 99% cure rate is possible only if sections 13–15 mm are taken [20].

One of the major advantages of MMS is its potential to allow the surgeon maximal normal tissue conservation. The surgeon evaluates the slides to determine if the margins are involved. If the tumor is completely excised the surgical defect is reconstructed. If tumor is present, the corresponding location on the map is marked. If the lateral margin is involved, an additional excision of 1–2 mm tissue is removed. If tumor is present in the deep margin, an incision is made along the inside of the defect's edges, and a thin strip of tissue is removed from the depth of the defect by scissor dissection for additional histologic evaluation. These stages are repeated until the margins are considered clear and the reconstruction can be performed.

H&E = hematoxylin and eosin

Depending on a variety of factors, MMS may paradoxically result in margins that are either too wide or too narrow [Table 2]; a middle ground between these extremes is probably the rule [1].

CONCLUSION

Since its introduction, the techniques used in MMS have continued to evolve. Despite these technical differences, the accuracy and meticulous skill applied at each step of the procedure leads to consistent and repeatable cure rates. MMS is performed by a team of surgeons, nurses and histotechnicians, whose cooperation and communication in excising handling, mapping, processing, and histologic examination of tissue specimens are crucial for ensuring the efficiency and integrity of this technique.

Table 2. Factors determining MMS margin width

WIDE MARGINS [1]

Excessive debulking prior to taking margins, taking thick layer for margins [21]

- Aggressive surgeon (desire to have 100% cure rate or "flapophilia")
- Patient demanding 100% chance of cure in one Mohs stage
- Infection, crusting or hyperkeratosis may cause exaggerated estimate of tumor size
- Ill-defined margins may result in a wider debulking layer taken
- Dermatitis (seborrheic or contact) around neoplasm makes it seem larger
- Actinic damage or keratosis around neoplasm that may appear to be part of the neoplasm
- Dense fibrosis (especially in recurrences) may obscure actual lesion size

Extra margin taken because of histologic finding in the margin

- Inflammatory cell aggregates [22]
- Keratin granulomas [22]
- Folliculocentric basaloid proliferation [23]
- Pseudocarcinomatous hyperplasia from previous excision [24]
- Squamous metaplasia of sweat glands or salivary glands [24]
- Single atypical melanocytes (not pagetoid) around melanomas [25]
- Granulation tissue or fibrosis resembling spindle-cell neoplasms [1]
- Tangential sections through adnexal structures that resemble neoplasm [14]
- Equivocal positive frozen section

Technical errors

- Mapping error
- Excessive facing of block produces false-positive margin
- Misoriented section (cutting wrong side)
- False-positive reading of frozen section (observer error)

NARROW MARGINS: BETTER COSMETIC, HIGHER "RECURRENCE" RATE [1]

Inadequate debulking prior to taking margins, or taking thin layers for margins

- Timid doctor, "flapophobia"
- Cosmetic considerations (young or beautiful patient, celebrity, or desire of patient or doctor for a smaller scar)
- Avoidance of anatomic structures (arteries, nerves, tendons)
- Previous excision site inadequately debulked, misleading negative margin obtained above or medial to plane of residual tumor
- Multifocal tumor (misleading negative margin), especially sebaceous carcinoma, Bowen's and Paget's disease and in recurrences where discontinuous foci may exist

Technical errors

- Mapping error
- Misoriented specimen
- Incomplete sections (holes in section or failure to observe the entire epidermal edge)
- False-negative reading of frozen section (observer error)

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Capsule

Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling

Toll-like receptors (TLRs) are crucial to innate immunity. Activation of these proteins, and of receptors for the pro-inflammatory cytokines interleukin (IL)-1 and IL-18, leads to the recruitment of adaptor proteins such as MyD88. These in turn interact with further proteins such as IRAK2 and IRAK4. The crystal structure of the MyD88-IRAK2-IRAK4 death domain complex is now reported by Lin and co-authors, explaining how these three proteins cooperate in TLR/IL-1R signaling. Formation of these Myddosome complexes brings the kinase domains of IRAKs into proximity for phosphorylation and activation. Composite binding sites are required for recruitment of the individual DDs in the complex, which are confirmed by mutagenesis and previously identified signalling mutations. Specificities in Myddosome formation are dictated by both molecular complementarity and correspondence of surface electrostatics. The MyD88-IRAK4-IRAK2 complex provides a template for Toll signalling in Drosophila and an elegant mechanism for versatile assembly and regulation of DD complexes in signal transduction.

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Eitan Israeli

"Try not to become a man of success but rather try to become a man of value"

Albert Einstein (1879-1955)

"Do what you can, with what you have, where you are"

Theodore Roosevelt (1858-1919), 26th President of the United States

Magnetic Resonance Imaging and Implantable Cardiac Electronic Devices: It's Not What We Can Do, It's What We Should Do

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KEY WORDS: imaging, implantable cardioverter defibrillators, magnetic resonance imaging, pacemaker, safety

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Magnetic resonance imaging is an invaluable medical diagnostic tool used for many common diseases and conditions. Currently, millions of patients around the world have implanted cardiac devices, namely, pacemakers or implantable cardioverter defibrillators. However, for many years these patients were prohibited from having MRI scans because their device may interact with MRI machines, potentially affecting the device or compromising patient safety.

One of the chief advantages of MRI it that it has no ionizing radiation. In order to generate an MR image three types of electromagnetic fields are used: a constant static magnetic field, a rapidly changing magnetic gradient field, and a strong radiofrequency field. Pacemakers and ICDs contain ferromagnetic components, complex electrical systems, and leads that are implanted into the myocardial tissue. As a result, several potentially hazardous events can occur: movement of the device, programming changes, asynchronous pacing, activation of tachyarrhythmia therapies, inhibition of pacing output, and induced lead currents that could lead to cardiac stimulation [1-3]. In addition, heating of the lead tip can result in tissue damage as well as changes in thresholds, with the potential loss of lead function.

According to estimates, at least half of all patients worldwide with implanted cardiac devices are expected to need an MRI scan during the lifetime of their devices [3]. This is certainly a burning question that the medical community will face more frequently in the coming years.

In the 1980s, severe adverse events occurred as a result of unknowingly scanning individuals with pacemakers [4,5]. With advances in technology and better electromagnetic interference protection, several devices were tested in vitro and in animals in the MR environment and were found safe [6-9]. No significant device malfunction occurred. More importantly, no tissue damage or change in threshold was observed, reinforcing the key role of heat dissipation by blood flow inside the heart. In parallel, in recent years, several prospective human trials reported the relative safety of MR examination at 0.5–3.0 Tesla field strength. Data on almost 500 patients who underwent clinically driven MRI are now available [10-16]. No deaths have been reported in physician-supervised MR studies in which the patients were carefully monitored. In only a few cases have there been reports of minor changes in pacing threshold, the need for device reprogramming, and possibly battery depletion.

In this issue of *IMAJ*, Halshtok et al. [17] add to the cumulative published data. They present their single-center experience with uneventful MRI scanning in 18 patients (11 pacemakers and 7 ICDs) and a total of 34 scans (1 patient underwent 11 scans). They conclude that MRI scanning in the presence of cardiac implantable devices is safe and feasible, although not recommended for routine scans. Our group has performed safe MRI scanning in more than 40 patients (49 MRIs) with both pacemakers and ICDs and we concur with Halshtok that in patients with cardiac implant devices, when clinically indicated, MRI may be performed under strict conditions.

There is a wide range of available MRI systems, MRI scanning conditions, patient positions, pacemaker and ICD systems, and leads. Consequently, it is not possible to test all the combinations and prove the safety of a particular system. Thus, extending these results to recommendations for routine use of MRI in these patients should be done with caution. The fact that hundreds of patients with pacemakers or ICDs underwent uneventful MRI does not allow us to conclude that MRI in this population is indeed safe. Just crossing a highway blindfolded ten times without getting hit by a car does not make it safe. The true number of patients who experienced adverse events during and/or after MRI is unknown because it has not been reported. All published studies were performed at centers with expertise in MRI and device monitoring and were limited to patients with a true clinical need for MRI.

Another implanted cardiac device used to monitor arrhythmia is the loop recorder, a small subcutaneously implanted programmable device that

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ICD = implantable cardioverter defibrillators

contains two surface electrodes. This device has no lead wires and has received approval from the U.S. Food and Drug administration for MRI. Clinical MR studies of patients with these loop recorders did not demonstrate any subjective symptoms experienced by patients, adverse clinical events, or damage to the devices. Of note, interrogation of the devices after MR revealed tachyarrhythmias and bradyarrhythmias recorded during the examinations that were believed to be artifacts [18]. Patients with a loop recorder (Reveal Plus ILR, Medtronic) can undergo MR examination any time after implantation, provided there is no reason to believe the device is not well implanted. Because of the theoretical risk of electromagnetic fields adversely affecting data stored by the device, all stored data should be downloaded before scanning.

A device may be either MR safe, which means that it causes no known hazards in all MR environments, or MR conditional, which means that the device has been demonstrated to pose no known hazards in a specified MR environment with specified conditions of use [1]. The loop recorder is MRI conditional, but current pacemakers and ICDs are neither.

Yet, there is some encouraging news. A pacing system (the Medtronic EnRhythm-MRI SureScan) was designed, tested and approved for use with MRI under specified scanning conditions [19]. An international clinical trial to test the safety and efficacy of this prospectively newly designed dual-chamber pacemaker and modified pacing leads has recently completed enrollment. The interim analysis was encouraging [20]. Accordingly, this device, the first MR conditional pacemaker, received the European CE mark. This system is now commercially available in several European countries and in Israel, and is currently under clinical evaluation in the United States.

So what should we do with the patient with a pacemaker or ICD who needs a brain or spine or knee MRI now? Our patients have devices that were implanted in the past and were not designed, tested or approved for use with MRI.

The current consensus is that MRI should be done only when there is a true need, and only MRI and no other imaging modality can help with the diagnosis. The diagnostic benefit from MRI must outweigh the presumed risks [1,2]. Faris and Shein from the FDA [3] state: "for some patients, the risks presented by MRI under specific, characterized scanning and monitoring conditions may be acceptable given the diagnostic benefit of this powerful imaging modality."

Position papers with guidelines were issued in Europe and North America with detailed background, possible hazards, available laboratory and human clinical data, and recommendations [1,2]. The risks of MR scanning should be discussed with the patient, and written informed consent must be obtained before MR scanning. The MR study should be performed at centers with expertise in MRI and electrophysiology. The MR scan should be optimally planned to minimize time and energy. A physician who is knowledgeable in device therapy and programming should preferably be present during the MR scan. Thoughtful pre-MR reprogramming, careful patient monitoring during MR scanning, and thorough follow-up after MR scanning must be performed. Full resuscitation facilities should be available in case of an adverse event.

In summary, it is not what we *can* do, it's what we *should* do. MR imaging in patients with pacemakers or ICDs can be performed, but it is an off-label procedure requiring sound justification and safety precautions. It should be performed only when clinically indicated and only in selected centers with the proper expertise, resources and experience. Individuals with a pacemaker or ICD can now benefit from the advantages of this imaging modality.

FDA = Food and Drug Administration

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Capsule

Cross presentation of some antigens requires an early processing

CD8+ T cells respond to infections by recognizing peptide antigens bound to major histocompatability complex class I (MHC class I) protein expressed on the surface of antigen-presenting cells (APCs). Because MHC class I can only present intracellular antigens, antigen cross-presentation is important for responses to viruses that do not directly infect APCs. Antigen cross-presentation occurs when APCs acquire antigens by phagocytosis of dying virally infected cells and present them to CD8+ T cells. After internalization, antigens must enter the cytosol for processing by the proteasome. Singh and Cresswell show that GILT (gamma-interferon-inducible lysosomal thiolreductase) is required for cross-presentation in mice of disulfide-containing viral antigens. In mice lacking GILT, the CD8+ T cell response to antigens derived from disulfide-rich proteins was substantially impaired after influenza A or herpes simplex virus 1 infection. Thus, cross-presentation of some antigens requires an early processing step prior to proteasomal degradation in the cytosol and subsequent MHC class I loading.

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Eitan Israeli

Capsule

Magnet for medical tourism

Israel has long enjoyed a reputation as a global leader in medical R&D and high-tech applications that save lives and improve quality of life. The high standards of health care have given rise to a phenomenon known as medical tourism. In 2009 one and a half million Americans went abroad as medical tourists. Even paying a portion of the bill (the insurance company pays the rest) the entire medical cost in some countries can still result in a net savings. For those who do not have health care insurance, traveling internationally is often the only way to receive necessary medical treatment. Israel is high on the list for many Americans. Many Israeli specialists were trained in the U.S. and are recognized as world-renowned authorities in their field, with pioneering techniques and high rates of success. Numerous American companies, in seeking better health care for their work force, have begun offering medical tours to Israel as part of their employee health insurance benefits. Among the attractions is the prospect of an all expense-paid vacation in a country with a rich history, Mediterranean climate and luxury hotels. For Americans with strong religious beliefs, the combination is compelling, with sites holy to Judaism, Christianity and Islam. One of the major options is IVF treatment. Couples who have not succeeded in having children can expect to pay over $20,000 for IVF in the United States (usually not covered by insurance), while the same treatment is available at 24 fertility clinics in Israel for $4000, in the care of specialists with one of the highest success rates in the world (35–40% compared to the global rate of 20%). All Israeli hospitals have medical tourism centers. The treatments are comprehensive and include orthopedics, oncology, cardiology, urology, cosmetic surgery and rehabilitation of injuries as well as treatment for obesity and infertility.

Israel High-Tech & Investment Report May 2010

"There's never been a true war that wasn't fought between two sets of people who were certain they were in the right. The really dangerous people believe they are doing whatever they are doing solely and only because it is without question the right thing to do. And that is what makes them dangerous"

Neil Gaiman (b. 1960), American novelist and short story writer

The Association between Mycoplasma Infections and Atherosclerosis: Myth or Clinical Reality?

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KEY WORDS: atherosclerosis, autoimmunity, Mycoplasma, coronary artery disease, myocardial infarction

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Atherosclerosis is a multifactorial disease with growing prevalence worldwide and is currently considered an immune mediated inflammatory disease [1]. Several autoantigens have been identified in atherosclerosis: heat-shock proteins, β2-glycoprotein-I, and oxidized low density lipoproteins. Increased inflammation in the atherosclerotic plaque is associated with increased plaque vulnerability [2].

Tissue damage due to infections, exposure to autoantigens, molecular mimicry, bystander activation of autoreactive immune T cells, and persistent infections may trigger autoimmunity [3]. Therefore, it is plausible that some infectious agents may trigger or aggravate immune mediated atherosclerosis. An association between infections and atherosclerosis was first demonstrated in 1978 [4]. Several infectious agents have been suggested as possible contributors to the acceleration of the atherosclerosis process [5]. Espinola-Klein and colleagues [6] reported that elevated antibody titers against *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus-2 and cytomegalovirus are associated with more advanced atherosclerosis, after adjustment for other risk factors. Although the association between some infectious agents and atherosclerosis are supported by several studies, a controversy still exists as to the possible role of *Mycoplasma pneumoniae* in induction and acceleration of atherosclerosis. Moreover, it is unknown whether an immune reaction against *M. pneumoniae* is associated with an adverse prognosis.

Animal model studies with *M. pneumoniae* have also yielded conflicting results. Intraperitoneal inoculation of *C. pneumoniae* and *M. pneumoniae,* or both, aggravated atherosclerosis in apolipoprotein-E knockout mice fed a cholesterol-enriched diet [7]. Nevertheless, in a rabbit model, *M. pneumoniae* was found to be associated with periaortitis, but not atherosclerotic lesions [8].

Using an electron microscopy apparatus, Higuchi et al. [2] were probably the first to detect *C. pneumoniae* and *M. pneumoniae* in thrombosed ruptured atheromas in humans. It has been suggested that large amounts of *C. pneumoniae* and *M. pneumoniae* in the plaque contribute to its vulnerability [9]. In 2006, Weiss and colleagues [5] evaluated the presence of *M. pneumoniae* (by polymerase chain reaction) in atherosclerotic plaques of the carotid artery, in apparently healthy greater saphenous veins and in circulating leukocytes. Samples were collected from 36 patients who had carotid artery stenosis and 25 without evidence of marked carotid artery stenosis. No association was found linking the presence of *M. pneumoniae* DNA in leukocytes, carotid plaques and veins with inflammatory markers [5]. In addition, Reszka et al. [10] found similar detection rates of *M. pneumoniae* in the aortic wall of patients with three-vessel coronary artery disease and in those who had normal coronary angiography and needed aortic valve replacement.

Momiyama et al. [8] used the complement fixation test to evaluate seropositivity for *C. pneumoniae* and *M. pneumoniae* in 396 patients with coronary artery disease, and 153 patients without coronary artery disease. Anti-*M. pneumoniae* antibody titer ≥ 1/8 and ≥ 1/16 were significantly more common in patients with coronary artery disease, although no difference was found for a titer of ≥ 1/4 (also considered seropositive) [8]. In a multivariant analysis, *M. pneumoniae* (titer of ≥ 1/8) was found to be associated with coronary artery disease only in patients seropositive for *C. pneumoniae* [8]. In addition, Goyal et al. [11] reported that combined seropositivity with *M. pneumoniae* and *C. pneumoniae* are more common in patients with coronary heart disease and a history of myocardial infarction. Reunanen et al. [12] found that the incidence of coronary artery disease in men without a prior history of heart disease significantly increased in those with the highest quartiles of antibody levels against *M. pneumoniae*, compared to men with antibody titers in the lowest quartile. Nevertheless, the presence of anti-*M. pneumoniae* antibodies did not demonstrate prognostic significance in men with a positive history of heart disease [12]. Maia and collaborators [4] reported a lack of statistical significance when values of anti-*M. pneumoniae* IgG

Ig = immunoglobulin

levels of patients with acute coronary syndrome were compared with the antibody titer of patients with chronic coronary disease and controls. Additionally, patients with chronic atherosclerosis had a similar antibody titer compared with controls [4].

In the current issue of *IMAJ*, Barski et al. [13], using an agglutination test or enzyme-linked immunosorbent assay, measured the anti-mycoplasmal antibodies in 150 patients with coronary heart disease and in 98 healthy blood donors, noting that patients with coronary heart disease do not have a higher rate of seropositive results for *M. pneumoniae, Ureaplasma urealyticum, M. fermentans, and M. hominis* compared with the controls. Barski et al. [13] provide the first systemic evaluations of the association between *Ureaplasma urealyticum, M. fermentans and M. hominis* – and coronary heart disease. The authors carefully noted that the results did not exclude the association between Mycoplasma infections and atherosclerosis and suggest that the intracellular localization may be associated with less pronounced humoral activation and antibody production.

Barski et al. [13] are in agreement with Espinola-Klein et al. [6] who found no correlation between the extent of atherosclerosis and seropositivity for *M. pneumoniae* IgG and IgA. Nevertheless, they reported a higher mortality in patients who were seropositive for more pathogens (including *M. pneumoniae* and others) and thus subject to a higher infectious burden [6]. In another study, Espinola-Klein et al. [14] reported an association between seropositivity for *M. pneumoniae* IgA and progression of carotid atherosclerosis in a univariant analysis. However, significance was obliterated following adjustment for other conventional risk factors.

Importantly, definite conclusions regarding the role of Mycoplasma infections in atherosclerosis and heart disease is limited by the different methodologies for evaluating infection (namely PCR, in situ hybridization, electron microscopy techniques, ELISA, indirect immunofluorescence, and complement fixation test), and different criteria for defining serologic positivity. It should be emphasized that anti-*M. pneumoniae* IgG measurement via different ELISA kits have a high sensitivity (75–83%), whereas measurement of IgM is highly dependent on the type of kit used (16–58% sensitivity) [15]. Csángó and and co-authors [16] also reported a wide range of detection frequency of anti-*M. pneumoniae* IgM (2.8–16%) and IgA (22.8–68.5%) in healthy blood donors when different kits were used, and concluded that the use of certain kits may lead to over-diagnosis. Moreover, no association was observed in small studies between anti-*M. pneumoniae* IgG and positive PCR test of atherosclerotic plaques and circulating leukocytes [5].

In conclusion, it seems unlikely that a single infectious agent is associated with atherosclerosis, coronary artery diseases and cardiovascular events [6]. Uncertainty still remains regarding the possible contribution of Mycoplasma infections to the process of atherosclerosis, especially in the presence of a high infectious burden. Future research should focus on establishing standards of measurements for detecting Mycoplasma infections, and evaluating the consequences of Mycoplasma co-infection with other specific pathogens.

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PCR = polymerase chain reaction
ELISA = enzyme-linked immunosorbent assay

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Mohs Micrographic Surgery: Revisiting its Definition

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KEY WORDS: Mohs micrographic surgery, skin cancer, surgeon, pathologist

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"The crucial idea of excising the cancerous site layer by layer and systematically examining the undersurface of each excised layer under the microscope by means of frozen sections is so logical that it is surprising that it was not thought of a century ago."

Dr. Frederick Mohs [1]

In this issue of *IMAJ*, Arnon et al. [2] review a surgical method for the removal of skin cancers, known as Mohs micrographic surgery. This method, and its variants, aims at destroying the skin cancer while affording a maximum degree of preservation for the healthy surrounding tissue, which results in better functioning of the affected organ and a better appearance. The essentials of MMS [3] were published by the American College of Mohs Surgery in 1988, and in 1994 the American Academy of Dermatology published a position paper on the guidelines of MMS [4]. This surgical method has been developed and performed by dermatologists, and most of the articles published in peer-reviewed journals were written by dermatologists. One of the basic features of MMS is the examination of tissues under the microscope by the surgeon himself, as cited in Cottel et al. [3]: "All frozen sections are evaluated microscopically by the Mohs micrographic surgeon and the location of any remaining tumor is marked by the Mohs micrographic surgeon on the tumor map." This basic feature, together with horizontally cut frozen sections and accurate tissue mapping are the distinguishing marks of this method. Or, in the words of the American Academy of Dermatology, "The Mohs surgeon normally acts in two integrated, but separate and distinct capacities: surgeon and pathologist" [4].

MMS has begun to spread among dermatologists worldwide, both in academic institutions and in private practice. In 1978 Shelly [5] suggested honoring Dr. Mohs by using his name not only as an eponym but also as an acronym for his technique, namely, **M**icroscopically **O**riented **H**istographic **S**urgery. Since MMS may not be readily available, alternative techniques and variations of MMS for examining the surgical margins of the tissue in skin cancers have been developed in different parts of the world, bearing different names, such as Slow-Mohs, 3-D Histology and, recently, in Israel, the Mohs-like. Since this method has been proven efficient and highly curative, why do we need variations? There are obvious reasons, such as a) to acquire the ability to diagnose difficult tumors in frozen sections as in Slow-Mohs, b) the need for pathologic assistance as in Mohs-like when there are not enough qualified Mohs surgeons, and c) there are physicians who simply regard their own variations as more advantageous, such as those who practice 3-D histology. Furthermore, having a method that has proven efficient and highly curative, why do we need variations or imitations with the same name? Instead of a direct answer, allow me to cite another dermatopathologist: "Within the general practice of medicine, I have some concerns about Mohs micrographic surgery and medical ethics. It has become clear that the public must be protected against unscrupulous practitioners who inappropriately define themselves as Mohs surgeons, but who do not have the necessary training or technologic support to do the job properly. The reasons for such practice are all too obvious" [6].

While serving as Secretary of the European Society of Micrographic Surgery, it was clear to me why the word Mohs was excluded from the name of the Society. In certain European MMS variations, the surgeon does not read and interpret the histologic slides during the surgery [7]. Rapini, coauthor of the article published in this issue of *IMAJ*, wrote elsewhere about the definition of Mohs surgery [8]: "I advocate a broader definition of Mohs surgery as a method of excising skin cancer in stages using meticulously mapped-out peripheral sections of the margins that completely encompass the neoplasm, resulting in maximal tissue conservation while assuring clear margins histologically."

In contrast to his views and this definition, and based on my personal experience with MMS for almost 20 years, I prefer to define Mohs surgery in a rigid functional way: peripheral margins must be cut at an angle of 45 degrees in most cases; frozen sections are by definition part of modern MMS; the frozen section laboratory must be adjacent to the operating room; and the surgeon should be the one who reads the slides. If not all of these criteria are met, then the surgical method should not be called Mohs surgery, nor should the

MMS = Mohs micrographic surgery

word "Mohs" be part of its name. I add more points in favor of Mohs surgery, which were addressed by McGovern and Leffel [9]. Unifying the roles of surgeon and pathologist assures fewer errors when performing histopathologic and clinical correlation for each patient. Separating the tasks between two physicians increases the errors in mapping and applying the Mohs map to subsequent stages. However, I am strongly in favor of seeking quality assurance via interaction with a dermatopathologist.

In my opinion, all published data by Mohs surgeons "speak the same language," so we can therefore use the same definition. I believe the literature still lacks a solid database on "Mohs Surgery" performed by a surgeon and a pathologist on site and based on frozen sections. Nevertheless, all methods used to treat skin cancer have the same objective: to help our patients the best we can. At the same time, we as physicians are obliged always to inform the patients of alternative treatments. The patient is the one who has to decide whether or not he or she wants Mohs surgery, Pseudo-Mohs surgery, Mohs-like surgery or whatever. True Mohs micrographic surgery simply works. It is a well-accepted method that satisfies both the patient and the dermatologist.

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Capsule

Oral multispecies biofilm development and the key role of cell-cell distance

Growth of oral bacteria in situ requires adhesion to a surface because the constant flow of host secretions thwarts the ability of planktonic cells to grow before they are swallowed. Therefore, oral bacteria evolved to form biofilms on hard tooth surfaces and on soft epithelial tissues, which often contain multiple bacterial species. Because these biofilms are easy to study, they have become the paradigm of multispecies biofilms. Kolenbrande et al. describe the factors involved in the formation of these biofilms, including the initial adherence to the oral tissues and teeth, cooperation between bacterial species in the biofilm, signalling between the bacteria and its role in pathogenesis, and the transfer of DNA between bacteria. In all these aspects distance between cells of different species is integral for oral biofilm growth.

Nature Rev Microbiol 2010; 8: 471

Eitan Israeli

Capsule

Racing to create a bionic eye

An Israeli-international team is developing a technology that could restore sight to millions. Damage to the retina is among the leading causes of vision loss in the developed world. This "bionic eye" relies on the brain's acquired ability to process visual data. As such, it will only provide a limited field of vision. Nano Retina is developing an implant that will replace damaged photo receptors in the eyes and provide gray-scale vision to a resolution of 1300 pixels for a the first generation of chips and 5000 pixels for the second generation. The company hopes to begin marketing its implant within 5 years. The Germany biomedical firm Retina Implant AG, meanwhile, recently reported the successful conclusion of a clinical trial involving 11 subjects who lost their sight due to retinitis pigmentosa. A tiny chip implanted underneath the retina enables light entering through the pupil to be converted into neural signals that are received by the brain. The chip is powered by a tiny external battery that is affixed behind the ear. Nano Retina engineers say their chip will enable users to identify facial features and to watch television. While both chips use a similar biological infrastructure, Nano Retina's battery will be charged wirelessly by a mini-laser attached to a pair of eyeglasses.

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Macrophage Activation Syndrome Induced by Etanercept in a Patient with Systemic Sclerosis

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KEY WORDS: systemic sclerosis, macrophage activating syndrome, scleroderma, etanercept

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Macrophage activation syndrome is a form of secondary hemophagocytic lymphohistiocytosis associated with rheumatic diseases, neoplasia and/or infection [1]. More frequent in children, it has recently been described in an increasing number of adults. Although pathogenetically unclear, MAS is characterized by excessive activation and proliferation of T cells and macrophages leading to an overwhelming systemic inflammatory reaction with a disproportionate release of tumor necrosis factor, interleukin-6 and other cytokines. MAS is often misdiagnosed as sepsis or the exacerbation of an underlying disease [1]. It consists of acute-onset, persistent high fever, neuropsychiatric changes, splenomegaly, hepatomegaly, lymphadenomegaly, reduced number of red cells, white cells and platelets, abnormal liver function, and prolonged thrombin and prothrombin times.

Systemic sclerosis is a chronic rheumatic disease in which cytokines play an important pathogenic role. Biological agents are used today for the treatment of rheumatic disease in children and adults. Infliximab and etanercept for treating systemic sclerosis yield beneficial results and few adverse effects. In a study of 18 patients etanercept appeared to be efficacious in ameliorating active inflammatory joint disease, and it was safe and well tolerated. Health assessment questionnaire scores indicated an improvement following the treatment [2]. On the other hand, patients with juvenile idiopathic arthritis on anti-TNF therapy have an increased incidence of MAS [4]. There have been only two adverse reports on MAS in patients with scleroderma, both related to infliximab and described as a secondary effect of the possibly associated infection that triggered the syndrome. Etanercept or infliximab blocks TNF or TNF receptors and could theoretically aid in the treatment of MAS, but reports are conflicting, with both benefits and adverse reactions described. Etanercept has been used for the treatment of macrophage activation syndrome [3].

We present a patient with systemic sclerosis in whom MAS was precipitated by etanercept, since it was the only new variable prior to the onset of MAS that could explain the onset of the syndrome and there was no other triggering factor

PATIENT DESCRIPTION

A 70 year old woman with tight, shiny and brittle skin as well as hair loss on her forearms, hands, ankles and feet was admitted with complaints of diffuse arthralgias and synovitis in the proximal interphalangeal and metacarpo-phalangeal joints, Raynaud's phenomenon and shortness of breath. She was on symptomatic treatment for several months and underwent an open lung biopsy subsequent to X-ray findings of interstitial increased markings. She was referred for evaluation. A skin biopsy showed increased collagen and absence of fat. Antinuclear antibody was positive while other serologic tests (anti-Ro, anti-LA, anti-RNP, anti-SN, anti-SCL-70, anti-Jo1, anti-DNA) were negative. The diagnosis of systemic sclerosis was based on the history and clinical findings. The patient denied a history of hypertension, alopecia, kidney disease or other illnesses, but was intolerant to non-steroidal medications. Physical examination showed blood pressure of 110/70 mmHg, pulse 72/min, 22 breaths/min, and weight 51 kg. She had sclerodermatous changes on the dorsum of her hands and proximal fingers and mild sinovitis in the interphalangeal and metacarpo-phalangeal joints. Fine rales were heard in both bases with no other important changes. She did not have cardiomegaly and heart sounds were regular and rhythmic with no murmurs or gallop. No pedal edema was detected and the rest of the examination was normal. Echocardiogram revealed a pulmonary pressure of 18 mmHg and mild pericardial thickening. Because of her intolerance to non-steroidal anti-inflammatory medication, prednisone 7.5 mg daily was given for her arthritis.

On reevaluation 3 weeks after the biopsy, she was asymptomatic and doing well and the physical examination was unremarkable. She was scheduled to

MAS = macrophage activating syndrome

TNF = tumor necrosis factor

begin a combined regimen of methotrexate and etanercept, but because methotrexate was unavailable she was only given etanercept 25 mg subcutaneously and was maintained on 7.5 mg prednisone. Intense pruritus developed on her upper extremities that lasted for 8 hours on the day of the injection. Seventy-two hours later she was admitted to the emergency room following 48 hours of nausea, vomiting and abdominal pain; a few hours before admission she developed cough with hemoptysis and was increasingly lethargic. She was febrile, hypotensive, tachycardic and tachypneic (blood pressure 90/60, heart rate 120/min, respiratory rate 40); she had mild jugular venous distension, lymphadenomegaly, a systolic murmur, bilateral rales, tenderness over the right upper abdominal quadrant, and edema in the lower extremities. Laboratory tests showed white blood cells $14.9x10^3$, hemoglobin 5.49 g/dl, hematocrit 21.9, platelets 10^3x10^3, blood urea nitrogen 73 mg/dl, creatinine 7.2 mg/dl, aspartate aminotransferase 183 mg/dl, alanine aminotransferase 96 mg/dl, erythrocyte sedimentation rate 40 mm, low fibrin level, high level of fibrin degradation products, elevated triglycerides 290 mg/dl, and sodium 130 mg/dl. A chest X-ray showed a new bilateral interstitial pattern. A mixed respiratory and metabolic acidosis was observed and she was admitted to the intensive care unit with multiorgan failure as evidenced by renal, liver and respiratory failure. Bronchoscopy demonstrated pulmonary hemorrhage. All cultures and samples of blood, urine, stool, pharynx and bronchial aspirates, which were taken and done repeatedly, were negative for fungi, bacteria and parasites.A bone marrow aspirate revealed multiple macrophages phagocyting cell elements and ferritin. Serum ferritin was 852 ng/ml (normal 4–283 ng/ml, Abbot Axsym Systems, (Abbott Labs, IL, USA) and increased to > 1800 mg/ml on the following days. A diagnosis of MAS was made and the patient was started on cyclosporine and high dose methylprednisolone (30 mg/kg). She required invasive mechanical ventilation and hemodialysis. She also developed melena and a clotting disorder with a non-measurable prothrombin time. There was bleeding from the venipunture sites. Intravenous immunoglobulin was added, with no response. The patient died after 12 days. Limited autopsy with the consent of the family showed changes in the skin consistent with late-stage systemic sclerosis; changes in the liver compatible with erythrophagocytosis, with severe small-droplet steatosis, lipofuscinosis and marked intranuclear vacuolization; interstitial pneumonic changes in the lungs associated with systemic sclerosis, with no evidence of recent hemorrhage or infection; and initial signs of hypertensive cardiomyopathy.

COMMENT

The disease course in our patient, a 70 year old woman, began with shortness of breath, skin changes and arthalgias, with no definitive diagnosis. Symptomatic therapy was initiated. After several months of disease a lung biopsy was performed. Evaluation by a rheumatologist based on the clinical, skin and lung biopsy findings led to the diagnosis of systemic sclerosis. Owing to her intolerance of non-steroidal drugs she was started on 7.5 mg prednisone for the arthritis. Prednisone at a low dose has not been shown to be contraindicated in scleroderma patients or to induce systemic sclerotic renal crisis. Evaluation at the time showed that her condition was stable with amelioration of the joint symptoms and no clinical or laboratory changes. She received etanercept, a TNF antagonist reported to have a beneficial effect on patients with systemic sclerosis; however, within a few hours she developed a non-specific pruritus and MAS.

In a previous report describing MAS in a patient with systemic sclerosis who was receiving infliximab, the MAS was thought to be triggered by an Ebstein-Barr associated viral infection. MAS is triggered by viruses, bacteria, *Mycobacterium*, fungi and other parasites for which various medications are given. Our patient, however, had no evidence of infection in any of the repeated cultures – blood, sputum, urine and stool. Although etanercept has been used successfully in the treatment of MAS [3], it failed in our patient and was even thought to be responsible for the MAS. Etanercept has been associated with triggering MAS in patients with juvenile rheumatoid arthritis, and reports show an increased incidence of MAS in patients on etanercept therapy [4]. The clinical manifestations of MAS include fever, general malaise, fatigue, and mood and behavior changes, severe neurologic deficits, as well as hepatosplenomegaly with abnormal liver function test, low fibrinogen, high level of acute-phase reactants, reduced number of red cells, white cells and platelets, elevation of triglycerides and marked elevation of blood ferritin levels. Macrophages phagocyting blood elements are present in the liver, spleen and bone marrow, and hemorrhage occurs. Our patient had all these features. None of the signs or symptoms could have been interpreted as a systemic sclerotic renal crisis. Her liver findings were consistent with the diagnosis of MAS, since hemophagocytosis was present.

CONCLUSIONS

Macrophage activating syndrome is often underdiagnosed because clinicians are unfamiliar with the diagnosis, especially in adults. Physicians should be more alert to this entity; there is much to learn about this syndrome and the clinician must be able to identify cases that might behave as MAS and might respond only to therapy directed at their triggering factors, or that need additional therapy with methylprednisolone, cyclosporine or etoposide, which is also used in the treatment of

MAS. This diagnosis should also be considered in all patients with autoimmune disease, especially if there appears to be a reactivation of the underlying disease with signs of multiorgan system failure, with or without the presence of viral, fungal or bacterial disease, or when new medications are given. Much has to be learned about the relationship of these triggering factors and the onset of MAS.

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Capsule

A source for NK cells for cell-based antitumor therapy

T cells develop in the thymus, where they proceed through several developmental stages, losing alternative lineage potential as they progress. The molecular regulation of this developmental process, however, is not fully understood. P. Li et al. (*Science* 2010; 329: 85), L. Li et al. (p. 89), and Ikawa et al. (p. 93) now identify expression of the zinc finger transcription factor *Bcl11b* as the earliest checkpoint in T cell development in mice. Genetic deletion of *Bcl11b* in developing T cells inhibited commitment to the T cell lineage. Under conditions that should have stimulated T lineage differentiation, *Bcl11b*-deficient T cell progenitors failed to up-regulate genes associated with lineage-committed T cells and maintained stem cell- and progenitor cell-associated gene expression. In both developing and committed T cells, loss of *Bcl11b* resulted in the generation of cells that resembled natural killer (NK) cells in both phenotype and function. These NK-like cells could be expanded easily in vitro and possessed antitumor cytotoxicity, but they did not exhibit cytotoxicity against normal cells and were not tumorigenic. Because T cells are much easier to obtain from human patients than NK cells, deletion of *Bcl11b* in T cells may thus provide a source of easy-to-grow NK cells for cell-based antitumor therapies.

Eitan Israeli

Capsule

Creating a new biological entity from scatch

The DNA sequence information from thousands of genomes is stored digitally as ones and zeros in computer memory. Now, Gibson et al. have brought together technologies from the past 15 years to start from digital information on the genome of *Mycoplasma mycoides* to chemically synthesize the genomic DNA as segments that could then be assembled in yeast and transplanted into the cytoplasm of another organism. A number of methods were also incorporated to facilitate testing and error correction of the synthetic genome segments. The transplanted genome became established in the recipient cell, replacing the recipient genome which was lost from the cell. The reconstituted cells were able to replicate and form colonies, providing a proof-of-principle for future developments in synthetic biology.

Science 2010; 329: 52

Eitan Israeli

"Courage is what it takes to stand up and speak, Courage is also what it takes to sit down and listen"

Sir Winston Churchill (1874-1965), British Prime Minister, statesman and orator, historian, artist, and laureate of the Nobel Prize in Literature

"You never really understand a person until you consider things from his point of view... 'til you climb inside of his skin and walk around in it (from *To Kill a Mockingbird*)"

Harper Lee (born 1926), American author, best known for her 1960 novel *To Kill a Mockingbird*

Novel Influenza A (H1N1) and Acute Encephalitis in a Child

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KEY WORDS: H1N1, influenza, encephalopathy, encephalitis

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Neurologic complications of seasonal influenza have been well described in past epidemics. Encephalitis and encephalopathy, seizures, as well as post-infectious encephalitis and Reye syndrome, constitute the majority of these complications. The role of neurologic complications in the morbidity associated with the novel H1N1 virus is yet to be outlined. We report here the occurrence of encephalitis caused by the novel H1N1 virus in a pediatric patient, who shares many features of the few previously reported cases [1-3].

PATIENT DESCRIPTION

A previously healthy, febrile 9 year old boy was brought by ambulance to the Emergency Department following a convulsive episode. He was found unresponsive yet stable by the paramedics, who gave him a single dose of midazolam during transfer. The boy's mother reported fever accompanied by headache, cough and a sore throat beginning the day before admission. Headache subsequently worsened, with episodes of vomiting and apathy. The seizures consisted of rhythmic jerks and loss of urinary continence that lasted 10 minutes and ceased spontaneously. This was his first convulsive episode, with no personal or family history of febrile convulsions or neurologic diseases.

This was an obese child with a body mass index of 37.6, who upon admission to the Emergency Department was drowsy and disoriented and in marked agitation. Temperature was 40.0°C, oxygen saturation was maintained on a non-rebreather mask, and blood pressure was within normal limits. Apart from his neurologic state, the patient was tachypneic, yet the rest of the examination was unremarkable

Blood count showed leukocytosis of 13,300 cells/μl with 83.4% neutrophils. Blood chemistry, gases, ammonia and liver function tests were within normal limits. C-reactive protein was elevated to 54.6 mg/L. Computed tomography demonstrated clouding of the ethmoidal and maxillary sinuses but no intracranial pathology. A chest radiograph was normal. Lumbar puncture yielded 21 lymphocytes and 12 neutrophils/μl, with normal glucose and mildly elevated protein levels. Empiric therapy with ceftriaxone, acyclovir and oseltamivir was initiated, and the patient was admitted to the pediatric intensive care unit. Nasopharyngeal swabs were positive for the novel H1N1 influenza virus. Subsequent results of direct cerebrospinal fluid microscopy and bacterial culture were negative as was serologic testing of the CSF for H1N1 influenza, herpes and enteroviridae. Blood bacterial culture and serology for West Nile virus were negative. Ceftriaxone and acyclovir therapy was therefore discontinued and the patient completed a 5 day course of high dose oseltamivir 150 mg twice a day.

The patient remained disoriented with a diminished level of consciousness even though he did not receive any sedatives or narcotics. An electroencephalogram showed a mild background disturbance consistent with the encephalopathic state. Gradual neurologic improvement emerged on the third day of hospitalization and continued until the patient regained normal neurologic status 2 days later. The patient was discharged on the sixth day of hospitalization having attained a full recovery, and with no apparent neurologic or other sequelae.

COMMENT

Our patient suffered from fever and an altered mental status lasting several days in addition to CSF pleocytosis and an EEG tracing indicative of encephalitis, thus fulfilling the Centers of Disease Control criteria for encephalitis [1]. A large study of past epidemics of seasonal influenza estimated a 10% incidence of neurologic complications among hospitalized children, while encephalopathy was observed in 1% of the seasonal influenza pediatric patients [4]. Estimates from the current H1N1 influenza pandemic are still lacking, although a retrospective case series of children at an Australian tertiary care center reported encephalopathy in 3 of 43 pediatric patients, a rate higher than would have been expected according to previous experience with seasonal influenza [2].

Considering the small number of reports, it is difficult to characterize the neurologic morbidity associated with the novel virus. Still, the cases of encephalopathy reported so far from the current

CSF = cerebrospinal fluid

EEG = electroencephalogram

H1N1 pandemic display several similarities. The patients are children and young adults. The onset of encephalopathy, and in some cases also seizures, occurs relatively early in the clinical course, and the condition is self-limiting and leaves no residua. The CSF may show mild pleocytosis with no other noteworthy abnormalities. No influenza or other virus was detected in the CSF of any of the patients reported. Neuroimaging did not show any intracranial abnormalities in most of the cases, and the EEG was typically compatible with an encephalopathic process. All the patients were successfully treated with oseltamivir, while some received the drug in combination with rimantadine [1-3]. These findings conform with data collected during previous influenza seasons in the western world [4,5].

In the present report, we describe the first case of encephalitis due to the novel H1N1 virus in Israel, thereby adding to the accumulating body of evidence depicting the nature of the current influenza pandemic. Nevertheless, clinicians are still faced with significant uncertainty when treating H1N1 patients and should be on the alert for unique and atypical presentations.

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Capsule

Erasing DNA methylation during germ cell differentiation

Epigenetic reprogramming of the mammalian genome, which involves the removal and replacement of the various regulatory epigenetic marks such as DNA methylation, occurs during germ cell differentiation and during early zygotic development. This process is also critical during the experimental generation of stem cells, but the factors and pathways that control epigenetic reprogramming are not well understood. Hajkova and co-authors investigated the erasure of DNA methylation during germ cell differentiation and during early zygotic development in the developing mouse and found that factors involved in the base excision repair (BER) pathway, which helps repair damaged DNA, were involved. Furthermore, inhibitors of BER resulted in the retention of DNA methylation in the zygote.

Science 2010; 329: 78

Eitan Israeli

Capsule

Genetic basis of adaptation for living in high altitude

Peoples living in high altitudes have adapted to their situation. To identify gene regions that might have contributed to high-altitude adaptation in Tibetans, Simonson et al. (*Science* 2010; 329: 72) conducted a genome scan of nucleotide polymorphism comparing Tibetans, Han Chinese, and Japanese, while Yi et al. (p. 75) performed comparable analyses on the coding regions of all genes – their exomes. Both studies converged on a gene, *endothelial Per-Arnt-Sim domain protein* 1 (also known as *hypoxia-inducible factor* 2), which has been linked to the regulation of red blood cell production. Other identified genes that were potentially under selection included adult and fetal hemoglobin and two functional candidate loci that were correlated with low hemoglobin concentration in Tibetans. Future detailed functional studies will now be required to examine the mechanistic underpinnings of physiologic adaptation to high altitudes.

Eitan Israeli

"What a strange narrowness of mind now is that, to think the things we have not known are better than the things we have known"

Samuel Johnson (1709-1784), British author, poet, essayist, moralist, literary critic, biographer, editor and lexicographer

Multiple Mononeuropathy Secondary to Thrombosis of the Vasa Nervorum in Primary Antiphospholipid Syndrome

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KEY WORDS: antiphospholipid syndrome, Hughes syndrome, peripheral neuropathy, multiple mononeuropathy, vasculopathy

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Antiphospholipid syndrome is an autoimmune disease characterized by recurrent thrombotic events, miscarriages and thrombocytopenia associated with antiphospholipid antibodies (anticardiolipin, lupus anticoagulant, and anti-beta-2 glycoprotein I). Primary APS is defined as the presence of antiphospholipid antibodies in patients with idiopathic thrombosis without, however, any evidence of autoimmune disease or other triggering factors. Involvement of the neuropsychiatric system in APS accounts for high morbidity and mortality, with cerebral vascular accident being the most common manifestation. Peripheral nervous system involvement is rare in APS [1], and distal, asymmetric, axonal polyneuropathy (multiple mononeuropathy) is extremely rare, found most commonly in systemic lupus erythematosus and in certain types of vasculitis such as polyarteritis nodosa and Churg-Strauss syndrome. In APS, such association is infrequent, and, to our knowledge, there have been no reports of primary APS with multiple mononeuropathy.

PATIENT DESCRIPTION

A previously healthy 25 year old woman was hospitalized in November 2004 for investigation of cyanosis of the second, third and fourth toes of the right foot, as well as cyanosis of the fifth toe of the left foot, associated with severe livedo reticularis and pain. The patient reported no systemic arterial hypertension, dyslipidemia, diabetes, or smoking. Her blood pressure was 110/70 mmHg. The results of both her blood workup and lipid profile were normal. Blood glucose was 81 mg/dl. Testing for primary thrombophilia revealed protein S levels of 61% (normal values 55–160%), protein C levels 79.4% (normal 65–145%), and homocysteine levels 13.5 μM (normal 5–15 μM). Testing for antinuclear, anti-double-stranded DNA, anti-extractable nuclear antigen, anti-Sm, rheumatoid factor, and anticardiolipin antibodies, as well as for cryoglobulins, was negative. A working diagnosis of livedoid vasculitis was established. Treatment consisted of 1200 mg/day pentoxifylline, 40 mg/day nifedipine, and 100 mg/day acetylsalicylic acid. The patient's condition improved slightly after starting treatment. Two months later, hyperchromic patches with ulceration appeared in the malleolar region of both legs. The patches were accompanied by necrosis and pain in the second toe of the right foot.

The results of the laboratory tests were as follows: immunoglobulin-G anticardiolipin antibodies (enzyme-linked immunosorbent assay) 54 GPL units, IgM anticardiolipin antibodies (ELISA) 28 MPL units; lupus anticoagulant was positive. Lupus anticoagulant was measured according to international guidelines using activated partial thromboplastin time (Diagnostica Stago, France) and diluted Russel's viper venom time (Trinity Biotech, Wiclow, Ireland). Complement component 3 was 101 mg/dl, complement component 4 was 17 mg/dl; serology for human immunodeficiency virus, hepatitis B and hepatitis C was negative; and venereal disease research laboratory test serology was negative. Due to the clinical profile of skin ulcers, as well as the positive result for antiphospholipid antibody, treatment was begun with 60 mg enoxaparin twice daily, followed by oral anticoagulation therapy with warfarin. International normalized ratio ranged between 1.3 and 1.6. Four months later (March 2005), the patient was again hospitalized because of pain in the legs and partial loss of flexion and extension of the right foot, accompanied by purple hyperchromic patches with ulceration in the distal region of the right leg. Physical examination revealed: degree of muscle strength upon dorsal flexion of the right foot (foot drop) = 1, decreased tactile sensitivity in the inferior half of the right leg, decreased Achilles reflex, and present and symmetric pulses. Electroneuromyography showed marked degeneration of the motor and sensory nerve fibers of the posterior and fibular tibial nerves, as well as marked degeneration of the sensory nerve fibers of the sural nerves, a profile consistent with predominantly distal, sensorimotor, asymmetric, chronic multiple mononeuropathy. Sural nerve biopsy was performed, which revealed fibrin thrombus in the vasa nervorum of various vessels, with no evidence of any inflammatory process. The INR at that time was 1.17.

APS = antiphospholipid syndrome

Ig = immunoglobulin
ELISA = enzyme-linked immunosorbent assay

INR = international normalized ratio

Cerebrospinal fluid analysis showed normal results. Other secondary causes of peripheral neuropathy, such as vasculitis of small and medium-sized vessels, diabetes, thyroid diseases, exposure to chemical agent, neurotoxic drug exposure and paraproteinemia, were unequivocally ruled out. In addition, serum levels of vitamin B12 were normal. A skin biopsy in the pre-tibial region demonstrated thrombotic vasculopathy affecting subcutaneous cellular tissue. The patient received pulse therapy with methylprednisolone for 3 days, followed by 40 mg/day prednisone, weaning being gradual. The symptoms ameliorated after the initiation of treatment with 60 mg/day subcutaneous enoxaparin every 12 hours, maintained with warfarin. INR ranged between 1.9 and 2.6. The levels of IgG anticardiolipin antibodies then were 12.8 GPM and IgM anticardiolipin antibodies 46.4 MPL; lupus anticoagulant test results remained positive. Antiphospholipid antibodies were measured by the same methods on all occasions. A diagnosis of primary APS was established. Anticoagulation was maintained with warfarin; 90 mg/day diltiazem, 250 mg/day chloroquine and 25 mg/day amitriptyline were given, due to persistent lower limb paraesthesia. The patient exhibited progressive healing of the skin lesions and reversal of neurologic symptoms over a period of 11 months. Only slight paraesthesia of the dorsum of the right foot remained

COMMENT

To our knowledge, this is the first report of primary APS with multiple mononeuropathy secondary to thrombosis of the vasa nervorum affecting a patient who responded to treatment with anticoagulants.

The clinical presentation of APS varies widely, and the disease might present with neurologic syndromes. Although ischemic cerebrovascular accident is the most common neurologic manifestation, other symptoms have been observed, such as chorea, migraine, dementia and polyneuropathies [2]. Early detection of APS requires a high degree of clinical suspicion, especially when thrombosis occurs in unusual sites or when nonspecific symptoms predominate in the clinical presentation.

Our patient presented clinically with livedo reticularis and cyanosis of the toes associated with recurrent ulcerative lesions and subsequently developed lower limb paraesthesia and muscle weakness. Electroneuromyography revealed multiple mononeuropathy, and sural nerve biopsy showed thrombosis of the vasa nervorum associated with antiphospholipid antibodies on more than one occasion, confirming the diagnosis of APS according to the criteria of Sapporo [2]. It should be noted that the secondary causes of peripheral neuropathy were ruled out.

The mechanism of nervous system involvement in APS is considered to be primarily thrombotic [3]. However, the pathogenesis is not yet fully understood. Certain evidence suggests that the cerebral endothelium is activated by antiphospholipid antibodies promoting procoagulant activity. However, it is not clear how these antibodies trigger thrombosis. There is evidence that antiphospholipid antibodies can bind to glial cells, myelin and neurons, deregulating their functions and causing an immediate pathogenic effect [3]. It is possible that the prothrombotic state associated with antiphospholipid antibodies is responsible for changes in the cerebral microcirculation as the principal cause of certain types of neuropsychiatric manifestations observed in APS, including convulsions and cognitive dysfunction [3].

Multiple mononeuropathy is an infrequent manifestation of APS, being a typical clinical manifestation of vasculitis of small and medium-sized vessels. The pathogenesis of peripheral neuropathy involves immune and vascular mechanisms [4]. The inflammation and lesion of nerves might be caused by autoantibodies or immune complex deposits, or might be directly caused by vasculitis or thrombosis of the vasa nervorum [5]. Erten et al. [1] described a case of ischemic peripheral neuropathy with axonal degeneration, evidenced by sural nerve biopsy, in a patient with catastrophic secondary APS. Jeruc and colleagues [5] reported the case of a 49 year old patient, with no clinical or laboratory evidence of autoimmune disease or systemic vasculitis, who developed multiple mononeuropathy due to vasculitis with anticardiolipin antibodies. Sural nerve biopsy revealed active necrotizing arteritis with transmural inflammatory infiltrate and thrombosis. In conclusion, the present report draws attention to the neurologic changes in APS, which, although rare, are part of the spectrum of clinical manifestations of the disease and account for high morbidity, especially if the diagnosis is delayed or treatment is not begun promptly.

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Acute Unilateral Sensorineural Hearing Loss due to H1N1 Infection

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KEY WORDS: unilateral sensorineural hearing loss, H1N1, prednisolone

IMAJ 2010; 12: 450

The etiology of sudden deafness remains unknown even though some studies suggest that it could be viral in origin. Idiopathic sudden sensorineural hearing loss (unexplained unilateral sensorineural hearing loss with onset over a period of 72 hours) has an estimated incidence of 5–20/100,000 persons per year [1]. Approximately 1% of cases of sudden sensorineural hearing loss are due to "retrocochlear" disorders that may be related to vestibular schwannoma, demyelinating disease, or stroke. Another 10–15% are due to Meniere's disease, trauma, autoimmune disease, syphilis, Lyme disease, or perilymphatic fistula. The remainder are idiopathic and almost exclusively unilateral [1].

We describe a 73 year old man who was admitted with H1N1 infection and developed acute right ear sensorineural hearing loss. This is the first case with acute hearing loss and H1N1 infection to be described.

PATIENT DESCRIPTION

A 73 year old man was admitted with acute gastroenteritis and low grade fever. He started to feel nausea and vomiting 2 days before admission. His daughter, a 30 year old obese woman with Down's syndrome, was admitted to the intensive care unit of the hospital 3 days earlier due to massive bilateral pneumonia and acute respiratory distress syndrome. A positive diagnosis of H1N1 infection was reached following polymerase chain reaction analysis (throat and nose swab cultures).

Because of the family relationship and the acute illness of the daughter a throat swab for H1N1 infection was taken also from the father, and Tamiflu® (Roche, Israel) 75 mg twice a day was started. Twenty-four hours later we received a positive diagnosis of H1N1 infection, and Tamiflu was continued for 5 days.

On the fourth day he complained of sudden hearing loss in the right ear and a sensation of fullness in the ear. The physical examination was normal, with a negative Romberg test and without nystagmus. A comprehensive audiologic assessment revealed a bilateral normal eardrum with a normal pure-tone air and bone conduction (Weber and Rinne tests). Treatment was initiated with high dose prednisolone (60 mg daily) and after 24 hours he began to hear again in the right ear and the sensation of fullness in that ear disappeared. Prednisolone was tapered gradually over the next few weeks and the patient felt well with no hearing disability.

COMMENTS

Acute sensorineural hearing loss can occur as a complication of viral illness such as mumps and herpes zoster or herpes simplex virus [2]. Epstein-Barr virus has been shown to be involved in sensorineural hearing loss [3], as was human immunodeficiency virus [4]. In a survey of serum samples, multiple agents were found in 24 of 49 patients with idiopathic sudden hearing loss. Influenza virus group B was found in 14 (18%) and rubeola in 12 (16%), followed by herpes simplex type 1 in 6 (8%), mumps in 6 (8%), influenza group A3 in 6 (8%), rubella in 5 (7%), and cytomegalovirus in 5 (7%) [5].

To the best of our knowledge our patient is the first to be reported with an acute unilateral hearing loss secondary to H1N1 infection. We believe that this phenomenon was related to the viral infection since it occurred during an acute viral infection and responded to high dose steroids within 24 hours of treatment.

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A NECESSARY STATEMENT

To the Editor:

Reading the excellent review presented by Yaron Niv, "Capsule endoscopy: No longer limited to the small bowel," in your March issue (2010; vol 12: p. 178), I searched the article for the necessary declaration regarding "conflicts of interest" as required by the International Committee of Medical Journal Editors (ICMJE) to whose requirements *IMAJ* is obligated. I did not find such a declaration. I quote: "As in the case of authors, silence on the part of reviewers concerning potential conflicts may mean either that conflicts exist and the reviewer has failed to disclose them or conflicts do not exist. Reviewers must therefore also be asked to state explicitly whether conflicts do or do not exist. Reviewers must not use knowledge of the work, before its publication, to further their own interests."

I suggest to the editors that they adapt the recommendations of the above mentioned committee, to the benefit of all concerned.

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To the Editor:

The comment of Dr. Nahum Werbin is important, since disclosure of any conflict of interest should be an integral part of any scientific paper. As a member of the Editorial Board of IMAJ, I support the notion of adding such a declaration for every paper accepted for publication.

Relating to the specific review that was chosen by Dr. Werbin to represent all the papers in IMAJ, I have two comments: I am glad that Dr. Werbin found it "excellent," and I have no conflicts of interest.

Yaron Niv MD
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Editor's note:

Readers are referred to our Instructions for Authors, which clearly stipulates the requirement for such a declaration.

CANCER SCREENING

To the Editor:

After reading the "First Report of Screening an Asymptomatic Population for Cancer," by Drs. Ben Boursi and associates (*IMAJ* 2010; 12: 21-5), I was left uncertain regarding some of the presented methods and findings.

First, Table 1 is entitled "Screening and surveillance recommendations" without stating the source of these recommendations. Professional organizations differ in their recommendations. There is undisputed evidence supporting early detection of breast, colorectal and cervical cancers. However, to the best of my knowledge, there is insufficient evidence to recommend for or against routine screening for the early detection of cutaneous melanoma, basal cell cancer, or squamous cell skin cancer, oral cancer, ovarian cancer, cancer of the prostate, testicular cancer and thyroid cancer.

Second, there seems to be an inconsistency between the caption of Figure 1 and the text. The figure says that colonoscopy, prostate-specific antigen, dermatologic examination, mammography, trans-vaginal ultrasound and Pap smear were "non-routine tests that were administered only when indicated." However, the text states that "All subjects had a thorough dermatologic examination; all men had a testicular exam; and all women had a trans-vaginal ultrasound and Pap smear.... The screening tests consisted of prostate-specific antigen for all men above the age of 40, colonoscopy for all men older than 40 and all women older than 50, mammography for all women over age 40." Therefore, it is not clear whether colonoscopy, prostate-specific antigen, dermatologic examination, mammography, trans-vaginal ultrasound and Pap smear were administered to all patients of the appropriate gender, or only when indicated.

Third, the authors state both in the text and in a footnote of Table 1 that "A low dose CT [was] performed only after discussing its value with the patients. Not all authorities recommend screening for lung cancer." This statement conveys the impression that all authorities do recommend screening for all of the remaining cancers, and this was further reinforced by the authors' statement that "There is little controversy about the potential importance of preventive measures in terms of lifestyle choices and early detection of cancer in reducing morbidity and mortality from cancer." I agree with the authors about the importance of lifestyle choices. However, I am not aware of any definitive evidence that early detections of cancer of the ovaries, uterus, skin, oral cavity, prostate, testicles and thyroid reduce mortality.

Fourth, the authors do not mention the frequency of false-positive findings. Table 2 indicates that benign or malignant tumors were detected in a total of 25 patients. Table 3 indicates that as many as 44 patients tested positive on any of the screening examinations. This suggests that 19 patients had a false-positive test result. How was malignancy excluded in these 19 patients? How many patients had additional investigations for false-positive test results? For example, Table 3 states that three patients had a positive test for prostate-specific antigen (PSA). Table 2 indicates that there were only two cases of prostate cancer. In other words, one patient had a false-positive PSA test. How was prostate cancer ruled out in this patient? By multiple biopsies? A second example: Table 2 indicates that there were no cases of lung cancer. Table 3 states that one patient tested positive on low dose CT. In other words, this patient had a false-positive low dose CT. How was lung cancer ruled out in this patient? By bronchoscopy? By thoracotomy?

Fifth, the report left me uncertain whether it describes a state-of-the-art practice or a research project. If it proposes a practice innovation, I miss its cost-effectiveness (how much money was invested in order to detect a malignant tumor?) and its evidence base. If this was indeed a research project, as suggested by the fact that the study was approved by the Tel Aviv Sourasky Medical Center Helsinki Committee, then I miss a conflict of interests statement: who funded the study? A grant donation? The hospital? The patient's health insurance? The patients themselves? Were the patients given any information in addition to the statement that not all authorities recommend screening for lung cancer?

Finally, I concur with the authors' conclusion that the benefit of early detection of cancer should be further studied. However, I am uncertain whether their report provides any evidence for such benefit, or for the efficacy of an Integrated Cancer Prevention Center devoted to early detection of cancer in apparently healthy adults.

Jochanan Benbassat MD
Brookdale Institute, Jerusalem

To the Editor:

We appreciate the comments of Prof. Benbassat and wish to provide the following clarifications and explanations.

1. Our study population comprised subjects aged 25–77, thus not all subjects had undergone colonoscopy, PSA or mammography. All subjects were first screened for dermatologic lesions by the physician at the center (N.A.), and in the event of a suspected finding they were sent to a dermatologist/plastic surgeon.
2. The author misunderstood the tables. According to Table 3, 29 lesions were detected among the subjects. Fifteen of those lesions were detected in average-risk subjects. Four lesions were not included in Table 2 since they were lesions with no malignant potential. Prof. Benbassat is correct with regard to the title of Table 3, which should have been "suspected neoplastic lesions."
3. Prostate cancer was ruled out by multiple biopsies. Regarding the patient with the lung lesion, the treating physicians decided to follow the CT finding and a repeated exam did not show a change in the lesion.
4. The current study is a pilot study which reports our initial experience with the first 300 subjects who underwent simultaneous screening for cancer. We needed the Helsinki Committee's approval for the genetics part of the study. The routine tests were partially paid by the patients who could afford it, or for free if they could not.
5. Time will tell. This is the first integrated cancer prevention center worldwide. We believe that from our initial observations better and more effective screening programs will be developed.

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Erratum
In the article "Clinical manifestations in Israeli cystinuria patients and molecular assessment of carrier rates in Libyan Jewish controls" published in *IMAJ* [2003; 5(6): 439-42], a mistake occurred in the spelling of one of the authors' name. It should be Kreiss Y and not Kreiss I as printed.

Capsule

Listeria monocytogenes and interferon

Intracellular bacterial pathogens, such as *Listeria monocytogenes*, are detected in the cytosol of host immune cells, where they induce a host response that is often dependent on microbial secretion systems. Woodward et al. show that *L. monocytogenes* produce and release cyclic diadenosine monophosphate into the host cytosol, which induces the production of host type I interferon. Because a number of intracellular pathogens contain the protein machinery to generate this nucleotide and also activate this same innate immune pathway, a common molecular mechanism may exist for host detection of cytosolic bacterial pathogens.

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Eitan Israeli

"Punishment is the last and least effective instrument in the hands of the legislator for the prevention of crime"

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

"Our character is what we do when we think no one is looking"

Anonymous