A Costly Covenant: Ritual Circumcision and Urinary Tract Infection

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ABSTRACT: **Background:** Ritual circumcision in neonates may cause a urinary tract infection within 2 weeks of the procedure.

Objectives: To evaluate the prevalence of urinary tract infection among Jewish male circumcised neonates (≤ 28 days old) evaluated for fever in the emergency room.

Methods: All available medical records of neonates presenting to the pediatric emergency room for evaluation of fever over a 10 year period were reviewed. Data included gender, ethnic background, age (in days) on presentation to the emergency room, age (in days) when circumcision was performed (in males ≥ 8 days of age), and results of urine, blood and cerebrospinal fluid cultures. Families of males older than 8 days of age who had a UTI were contacted by telephone to verify the circumcision status when the infant presented to the ER, to ascertain whether the circumcision had been performed ritually by a *mohel** or by a physician, and, if not recorded in the chart, to verify the day of life on which circumcision was performed.

Results: Among neonates older than 8 days of age, 60 (24.7%) of the 243 febrile Jewish males had a UTI, as compared to 12 (8.4%) of 143 females ($P < 0.0001$). In 39 of 54 male neonates (72%) for whom circumcision was performed ritually on the eighth day of life, UTI occurred within 9 days of the circumcision. For females, there was no such clustering of UTI cases in the second week of life, nor during any other time period.

Conclusions: Febrile male neonates who have undergone ritual circumcision have a high prevalence of UTI and must be evaluated and treated accordingly

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KEY WORDS: urinary tract infection, ritual circumcision

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One of the medical benefits of circumcision in newborn males is its protective effect against urinary tract infection, particularly in infancy. This was first recognized in 1982 [1] and subsequently substantiated in several well-designed studies [2-5]. Yet, traditional Jewish circumcision performed by a *mohel* has been associated with various complications [6-8], chief among them an increased risk for UTI in the first weeks following the procedure [9-13]. Since virtually all Jewish males in our patient population undergo ritual circumcision on the eighth day of life, we sought to determine the association between this procedure and the occurrence of UTI among male neonates with fever.

**A mohel* is a Jewish man trained in the practice of *Brit milah* (circumcision).

UTI = urinary tract infection
ER = emergency room

PATIENTS AND METHODS

A study we conducted on the prevalence of serious bacterial infection among febrile neonates who fulfill specific "low risk criteria" for sepsis was recently published [14]. The data assessed in this current study were part of the data collected for the previous one, but were neither analyzed nor reported in that publication. All available medical records of neonates (≤ 28 days) presenting to the pediatric emergency room of Shaare Zedek Medical Center for evaluation of fever during the 10 year period from June 1997 through May 2006 were reviewed. Neonates with a rectal temperature of ≥ 38°C measured in the emergency room, or at home prior to arrival, were eligible for this study. All neonates were evaluated for the presence of a serious bacterial infection including UTI. All urine culture specimens were obtained by bladder catheterization or suprapubic aspiration. The following data were collected from the chart of each neonate:

- Gender, ethnic background, age (in days) on presentation to the ER; and for circumcised males, age (in days) when ritual circumcision was performed.
- Results of the urine culture.
- Results of the blood and cerebrospinal fluid cultures among infants with a positive urine culture.

Since the medical records of many of the male neonates did not state whether or not the child underwent circumcision by a mohel on the eighth day of life, a telephone survey was conducted to ascertain this information from the families of the males who had a UTI.

Urinary tract infection was diagnosed if there was: any growth of a single known urinary bacterial pathogen, isolated growth of > 1000 colony-forming units/ml of a single skin

bacteria, > 1000 cfu/ml of at least one known urinary bacteria pathogen if two bacteria were isolated, or > 10,000 cfu/ml of at least one known urinary pathogen if three organisms were isolated [15].

STATISTICAL ANALYSIS

Data were typed into a computerized questionnaire written in Epi-Info 6.04d and analyzed by this program and by Epi-Info 3.5.1 (both from the Centers for Disease Control, Atlanta, USA) and PEPI for Windows (Abramson JH & Gahlinger PM, www.brixtonhealth.com). We applied the *t*-test for continuous variables, and the chi-square test (Fisher exact where applicable) for categorical variables. Approval for this study was granted by the Helsinki Committee of Shaare Zedek Medical Center

RESULTS

There were 449 febrile neonates who presented to the ER during the study period for whom complete medical records were available for analysis. Of them, 290 (65%) were males. UTI was found in 67 (23.1%) of the 290 males and 15 (9.4%) of the 159 females ($P \leq 0.001$). Among neonates older than 8 days of age, 60 (24.7%) of the 243 febrile Jewish males had a UTI, as compared to 12 (8.4%) of 143 females ($P < 0.0001$). Ritual circumcision was verified as having been performed on the eighth day of life in 54 of the 60 males. Of the 54 males, 52 had growth of a single organism and 2 had growth of two organisms. Of the 12 females, 10 had growth of one organism, 1 had two and the other had three organisms. The distribution of bacteria isolated from the urine of the 54 males and 12 females is shown in Table 1. When more than one organism was isolated, the bacteria with the predominant growth were listed. As shown in Figure 1, among the 54 male neonates, 27 (50%) of the UTI cases occurred within 5 days subsequent to their circumcision, and 39 (72%) within 9 days following circumcision. For females, there was no such clustering of UTI cases in the second week of life, or during any other time period. Trend analysis for UTI among males showed two-tailed $P < 0.002$ by Mann-Kendall, Kendall's tau and Spearman's rho tests.

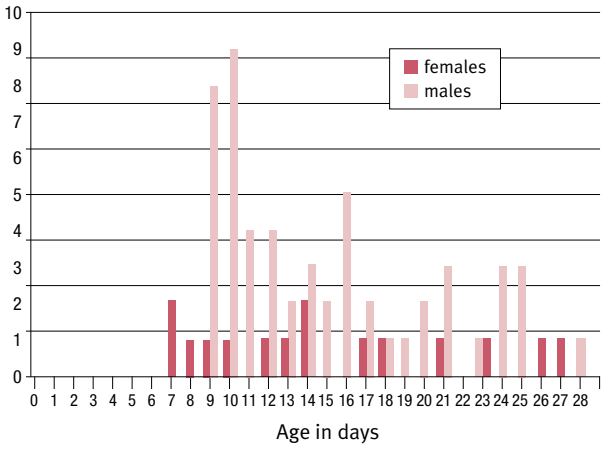
Importantly, 12 (10 male, 2 female) of the 82 neonates (14.6%) of all ages with UTI were bacteremic with the same organism that was isolated from their urine. Two of the neonates (both males) with UTI and bacteremia also had meningitis, with growth of the same organism in the CSF as was isolated from their urine.

cfu = colony-forming units

Table 1. Bacteria isolated from urine of the 66 neonates (> 8 days) depicted in Figure 1

Bacteria	No. of cases (%)
E. coli	46 (70)
Klebsiella	7 (11)
Enterobacter	3 (5)
Group B *Streptococcus*	3 (5)
Citrobacter	2 (3)
S. aureus	2 (3)
Enterococcus	1 (1)
Proteus	1 (1)
Pseudomonas	1 (1)

Figure 1. Distribution of UTI cases among 54 males circumcised on the 8th day of life, and all females



DISCUSSION

In this study we found that febrile male neonates who underwent Jewish ritual circumcision were significantly more likely to have a UTI than their female counterparts. Approximately one-quarter of all Jewish neonates who presented to the ER with fever had a UTI. In the majority of cases the UTI occurred within 9 days following the circumcision.

Our findings are consistent with those of other studies performed in Israel that also found an increased incidence of UTI following Jewish ritual circumcision, with most occurring between 1 and 12 days after [9-13,16]. *Escherichia coli* was the causative organism in the majority of cases in most of the studies, followed consistently in frequency by *Klebsiella.* The data from these various studies are concisely summarized in Table 2. As shown in Table 2, the time period following circumcision during which the incidence of UTI peaks was shorter than reported in other studies.

Recently, Prais et al. [13] retrospectively analyzed the incidence of UTI during the neonatal period among 87 males circumcised by a mohel according to Jewish ritual custom, as

CSF = cerebrospinal fluid

Table 2. Clinical features of male neonates with UTI following ritual circumcision

Author, year [ref]	No. of infants	Percentage of UTIs due to *E. coli* (E) and *Klebsiella* (K) respectively	Range in days of post-circumcision period	No. (%) of UTI cases occurring during post-circumcision time range	No. (%) of UTI cases attributed to circumcision with bacteremia
Amir et al, 1984 [9]	8	E - 88 K - 25*	0–17**	8 (100)	3 (38) **
Cohen et al., 1992 [10]	32	E - 67 K - 18	1–12	27 (84)	2 (7)
Goldman et al., 1996 [11]	20	E - 43 K - nr	2–13	14 (70)	4 (29)
Harel et al., 2002 [12]	49	E - 84 K - 13‡	1–22	nr	10 (18)‡
Prais et al., 2009 [13]	42	nr	1–22	nr	nr
Present study	54	E - 70 K - 11	1–9	39 (72)	9 (17%)§

*One urine culture had growth of both organisms

** Reference 16

‡ Out of a total of 55 infants, 6 were circumcised by a physician

§ One of these nine neonates had meningitis with the same organism isolated from the CSF as from the blood and urine

nr = not reported.

compared to 24 males circumcised by a physician according to standard medical practice. They found that 48% in the former group and 25% in the latter developed a UTI, rendering infants circumcised by a mohel 2.8 times (95% confidence interval 1–9.4) more likely to develop a UTI during the neonatal period than infants circumcised by a physician. Harel and colleagues [12] assessed the circumcision technique performed among 55 neonates who subsequently developed a UTI compared to a combined control group of 160 healthy young infants. In this prospective study the authors detected an even greater risk for UTI among neonates circumcised by a mohel compared with those circumcised by a physician, with an odds ratio of 4.34 (95% CI 1.62–12.27). The method of achieving hemostasis varied between the two practices, with the former using a gauze dressing wrapped around the penile shaft, and the latter the application of brief local pressure, calcium-sodium alginate fiber, and a wound cavity dressing that disintegrates within 2–3 hours. Importantly, the mean duration of hemostasis was found to be longer among neonates with UTI than among the control group without (25.6 ± 21.8 vs.16.6 ± 12.7 hours, P = 0.007). Urinary retention caused by gauze pressure, as well as post-circumcision periurethral colonization worsened by the shaft wrapping were therefore suggested as the mechanisms for the development of UTI following ritual circumcision. Previously suggested mechanisms for UTI occurring subsequent to ritual circumcision include non-sterile technique and pain-induced urinary retention [11].

CI = confidence interval

Since in our patient population virtually all males undergo circumcision on the eighth day of life, we were unable to assess UTI incidence in a control group of uncircumcised males. However, data from a large and seminal study by Wiswell et al. [2], which compared the incidence of neonatal UTI among circumcised and uncircumcised males, lend support to the notion that ritual circumcision causes UTI. Of the 100,157 males circumcised by a physician in U.S. Army hospitals, only 20 (0.02%) developed a UTI during the neonatal period. Neonatal UTI was 12 times more common among the 35,929 uncircumcised males in this study, occurring in 88 (0.24%) of such infants. The strong preventive effect of circumcision by a physician against UTI, which this and other studies [1,3-5] have demonstrated, further highlights the phenomenon that we and others [9-13] have described. Not only does ritual circumcision not have a protective effect against UTI in the neonatal period, it appears to have a causative one.

Furthermore, in the study by Ginsburg and McCracken [1], among the 62 male infants with UTIs, 95% of whom were uncircumcised, there were 29 whose UTI emerged between 7 and 29 days of life. The UTI cases among these neonates occurred with an even time distribution over the 3 week period. By contrast, the clustering of UTI cases between days 1 and 9 after ritual circumcision was performed on the neonates in our study points to the procedure as the likely culprit.

Fever in neonates is a relatively common event and may herald a serious bacterial infection such as UTI. As seen in our study, UTI in a very young infant may be particularly dangerous due to the possibility of resultant bacteremia and meningitis.

The incidence of bacteremia associated with post-circumcision UTI among the different studies in Table 2 varies between 7% and 38%. Though meningitis was not reported in the other studies, it is a known, albeit relatively rare complication of neonatal UTI [2,17] and is almost always associated with bacteremia. The potential for these two life-threatening complications associated with neonatal UTI further emphasizes the importance of early diagnosis and treatment of this condition.

A particular limitation of this retrospective study is the potential for error in recall by parents who were contacted in the telephone survey, since in some cases several years had elapsed since the child's circumcision. Nonetheless, even with the passage of time, it would be expected that Jewish parents would correctly remember the day of life on which their son was circumcised, and if the circumcision was performed by a mohel or physician. Other limitations are the lack of a control group of non-circumcised neonates, which we have previously addressed, as well as the lack of a control group of neonates circumcised by a physician. The study has a relatively small number of subjects, though combined with those presented in other similar studies adds further strength to the association between ritual circumcision and neonatal UTI.

Clinical practice guidelines have long recommended empiric intravenous antibiotic treatment and hospitalization for all febrile neonates [18,19]. However, a recent study performed in another region of Israel concluded that in well-appearing febrile neonates who fulfill specific "low risk" criteria, this may not be necessary since the rate of serious bacterial infection, including UTI, is extremely low [20]. We suggest that particularly for febrile male neonates who have undergone ritual circumcision, the significantly increased risk for UTI mandates a high index of suspicion and empiric intravenous antimicrobial therapy in the hospital. Further, the medical community should attempt to change the hemostasis technique used after ritual circumcision. It is likely that this would significantly reduce the incidence of a preventable serious bacterial infection in male neonates.

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Capsule

Influenza virus survives better at low humidity

One sneeze and influenza is drifting through the air, plastered across palms of hands and around door handles, poised for its next victim. How long can the virus survive outside a living host? The answer to this question depends on ambient environmental conditions. Shaman and Kohn (*Proc Natl Acad Sci USA* 2010; 106: 3243) showed experimentally that low absolute humidity (grams of water per cubic meter of air), which tends to prevail during temperate winters, improves the airborne survival of influenza viruses within aerosolized drops and favors transmission. Shaman et al. (*PLoS Biol* 2010; 8, e1000316) modeled how changes in absolute humidity have driven the seasonal peaks and troughs of influenza in the United States during a 30 year period. Epidemics were correlated with the onset of anomalously low absolute humidity, and variations in absolute humidity affected the occurrence of outbreaks during any one season. Thus, it may be just as feasible to forecast short-term influenza risk as it is the weather.

Eitan Israeli

"The thing I hate about an argument is that it always interrupts a discussion"

G.K. Chesterton (1874-1936), English writer, known as the "prince of paradox." His prolific and diverse output included philosophy, ontology, poetry, play writing, journalism, public lecturing and debating, biography, Christian apologetics, fantasy and detective fiction.

Prevalence of Celiac Disease in an Adult Jewish Population in Israel

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ABSTRACT: **Background:** In the last decade the frequency of celiac disease diagnosis has increased in adults.

Objectives: To determine disease prevalence (including silent and potential disease) in this population group.

Methods: We performed serologic screening of celiac disease in a representative and homogenous sample of a young adult general population in Israel, namely, 18 year old military conscripts, in 2003. Serologic screening was performed on serum samples randomly obtained from 850 healthy recruits (male/female = 1.1). Immunoglobulin A anti-tissue transglutaminase was determined by enzyme-linked immunosorbent assay. In cases of IgA deficiency, IgG anti-endomysial antibodies were determined. A small intestinal biopsy was offered to all patients with positive serology.

Results: The prevalence of overt CD diagnosed prior to recruitment was 0.12% (0.1% in men and 0.14% in women). The overall prevalence based on positive serology was 1.1%. Six of nine subjects with positive serology agreed to undergo endoscopy and intestinal biopsies. In all cases, biopsies were compatible with celiac disease (five biopsies were graded as Marsh 3a and one as Marsh 3b). One subject previously reporting irritable bowel-like symptoms was diagnosed with overt atypical CD. The prevalence of overt CD diagnosed by screening was 0.12%. The ratio of overt to silent CD was 1:8. No cases of potential CD were encountered.

Conclusions: Our findings suggest that CD is highly prevalent in the young adult population in Israel. Serologic screening for CD is a reliable and simple method for diagnosing this disease before symptoms or complications develop.

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KEY WORDS: celiac disease, prevalence, screening, young adult population

***Eran Israeli and Tiberiu Hershcovici contributed equally to the manuscript and share first authorship.**

Ig = immunoglobulin
CD = celiac disease

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Celiac disease is a permanent intolerance to dietary gluten characterized by an immune-mediated inflammatory lesion of the intestinal mucosa; a gluten-free diet assures full recovery. Due to its variable manifestations and age at onset, CD has emerged as a worldwide public health problem [1].

Although the 'classical' malabsorption syndrome characterized by diarrhea, steatorrhea, weight loss and fatigue may occur in severe cases, nowadays most patients present with a milder constellation of symptoms, such as abdominal discomfort and bloating mimicking irritable bowel syndrome, non-gastrointestinal symptoms (such as anemia and osteoporosis), or no symptoms at all. Due to this 'atypical' presentation, which is particularly frequent in the adult population, many patients are not diagnosed early. Prompt diagnosis and treatment of CD is associated with symptomatic improvement, reduction of potential complications (including malignancy), and decreased mortality [2,3]. It is therefore necessary to increase the awareness of medical professionals to the variable manifestations of CD; detection can be achieved by active screening.

The prevalence of CD varies greatly across different countries. This variability reflects population differences in the risk of CD, as well as differences in study design (e.g., serologic screening vs. symptom-based diagnosis, screening of general vs. high risk group populations). With these limitations in mind, the prevalence of CD in western populations (based on serologic screening) appears to be approximately 1%, with a reasonable range of 0.71%–1.25% [4]. However, the prevalence of CD is lower in other parts of the world such as South America [5] and Asia [6], whereas the disease rarely affects people of purely Chinese or Japanese origin. Since the origin of the Jewish population in Israel is diverse, we conducted a population-based study to determine the prevalence of CD including symptomatic and silent disease. The aim of this study was twofold: a) to determine the prevalence of symptomatic CD that was previously diagnosed, and b) to determine the prevalence of silent CD in the young adult population (aged 18 years) in Israel by active serologic screening.

PATIENTS AND METHODS

The prevalence of previously diagnosed CD was determined in the 18 year old population of military recruits during 2003 through the medical database of the Israel Defense Forces. Since military service is mandatory in Israel, the survey provides a representative sample of the young adult Jewish population. This population as a whole undergoes medical evaluation prior to recruitment to military service, independent of previously established medical diagnoses. Excluded from the survey are ultra-Orthodox Jews and Arabs, who are largely exempted from service and are thus under-represented.

Serologic screening was performed on serum samples from a representative sample of 850 healthy recruits. These samples were drawn from an ongoing, large-scale prospective survey on medical status, health behavior and attitudes routinely carried out among a fixed proportion of IDF recruits upon induction, 95% of whom are aged 18. The selection process for the survey is systematic and includes both male and female recruits based on a code calculated from the subjects' serial numbers. The selected serum samples were drawn from our serum bank for laboratory testing, and subjects' prerecorded demographic data were accessed from our computerized database. The study was approved by the IDF Institutional Review Board, and written informed consent was obtained from all selected recruits prior to entry into the survey.

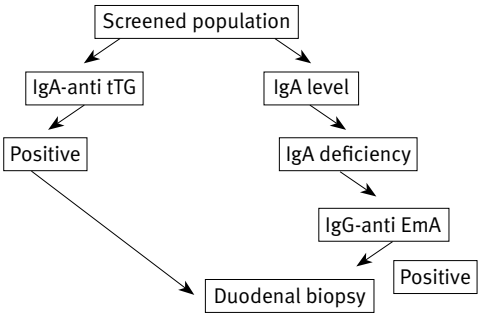
The medical history of all the subjects with a positive serology was assessed based on medical records, and the subjects were questioned with special focus on gastrointestinal symptoms and autoimmune diseases. A complete physical examination including body weight and height measurement was performed.

STUDY DESIGN

Blood samples were drawn from the antecubital veins of study participants on the day of recruitment and were stored at room temperature for up to 1 hour. Samples were then refrigerated for up to 2 hours at 4–8°C and were centrifuged for serum separation. Serum was then frozen at -20°C and stored at the IDF Health Surveillance Serum Bank until analysis. The screening algorithm is illustrated in Figure 1.

All samples were tested in parallel for IgA anti-tissue transglutaminase and total serum IgA level. IgA anti-tTG was determined by using a quantitative automated enzyme-linked immunosorbent assay method based on recombinant human tissue transglutaminase as antigen (EurospitalSpa, Trieste, Italy). Serum values of IgA anti-tTG higher than 7 AU/ml were considered positive. Total serum IgA level was determined by a radial immunodiffusion test. In cases of IgA deficiency, IgG anti-endomysial antibodies were determined by an immunofluorescence method on primate esophagus substrate (IMMCO Diagnostics, Buffalo, New York).

Figure 1. Flow chart of the study



Additional laboratory workup was done for serologically positive subjects, including a complete blood count, liver enzymes, serum albumin, ferritin, iron saturation, calcium and folic acid.

A small intestinal biopsy was offered to all subjects who were serologically positive. Four endoscopic biopsies from the second and third duodenal portions were obtained, fixed in 4% formaldehyde and embedded in paraffin. Specimens were stained with hematoxylin-eosin for morphologic assessment. The biopsies were assessed by two independent expert gastrointestinal pathologists and were staged according to the Marsh criteria as revised by Oberhuber et al. [7]. The diagnosis of intraepithelial lymphocytosis was made when more than 25 intraepithelial lymphocytes per 100 epithelial cells were observed.

RESULTS

The prevalence of overt CD diagnosed prior to recruitment in the entire population of military conscripts in Israel during 2003 was 0.12% (0.1% in men and 0.14% in women).

SCREENING OF ASYMPTOMATIC SUBJECTS [FIGURE 2]

Positive IgA anti-tTG was found in 1.1% (95% confidence interval 0.46–1.74%) of our study cohort (male/female ratio 1.25). Four patients had IgA deficiency. All these patients tested negative for IgG anti-EmA.

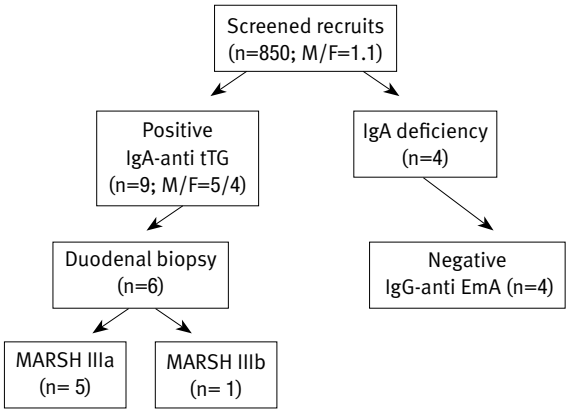
Six of nine subjects with positive serology agreed to undergo endoscopy with small bowel biopsies. All of these biopsies demonstrated duodenal villous atrophy. Five patients had partial villous atrophy (compatible with Marsh 3a) and one had subtotal villous atrophy (compatible with Marsh 3b). Based on these data, the prevalence of CD in our population was at least 0.7% (95% CI 0.44–0.96%).

Eight patients with positive serology were clinically asymptomatic and had normal laboratory results. One addi-

IDF = Israel Defense Force
Anti-tTG = anti-tissue transglutaminase

IgG anti-EmA = IgG anti-endomysial antibodies
CI = confidence interval

Figure 2. Flow chart of diagnostic steps according to the study design



tional patient was underweight and reported symptoms of abdominal pain and loose stools. This patient had Marsh 3b histologic grading and a normal laboratory workup. This subject had a previous diagnosis of inflammatory bowel syndrome, and the diagnosis of overt atypical CD was established by screening. Serologic screening established the diagnosis of overt CD in 0.12% of our cohort.

DISCUSSION

All military conscripts to the IDF undergo medical inquiry and screening prior to their recruitment; they represent the vast majority of the Jewish population of Israel. The process of recruitment is unique to the IDF; it provides the opportunity to examine the prevalence of a particular disease that was diagnosed before recruitment. The IDF sera database was utilized to screen a representative and homogenous sample of the young adult general population in Israel for the same disease.

We chose IgA anti-tTG as the preferred screening test, based on recommendations from a recently published National Institutes of Health consensus [8]. This test has a high sensitivity and specificity (similar to IgA anti-EmA), is easier to perform, is less observer-dependent and less costly than the anti-EmA test and is therefore more suitable for large screening programs [9].

In our study the prevalence of previously diagnosed subjects with overt CD was 0.12% and is similar to that found in a regional study previously carried out in the southwestern region of Israel (0.17%) [10]. This prevalence is in accordance with the literature data from both the United States and Europe (0.02% to 0.27%) and is much lower than the prevalence of asymptomatic disease [11,12].

Clinical CD represents the tip of the iceberg [13-15]. According to our findings, the prevalence of biopsy-proven CD diagnosed by screening is at least 0.7%. We calculated a positive predictive value of 29% for positive serologic testing based on the prevalence determined by our study (0.7%) and the sensitivity and specificity of the IgA anti-tTG [4]. Based on this figure one of the three subjects who refused endoscopy would likely have had an abnormal biopsy categorizing him as silent CD. Thus, the true prevalence of CD (including overt and silent CD) detected by screening is likely to be even higher, approximated as 0.9%.

The present study demonstrated that the prevalence of serologically diagnosed CD is higher than previously found in healthy blood donors in Israel (0.6%) [16], but similar to its prevalence worldwide [17-19]. Several reasons for the higher prevalence in our study compared to the study of Shamir et al. [16] are plausible First, CD is more common in women, who were under-represented among the blood donor cohort. Second, CD subjects with anemia may not have been allowed to donate blood.

In our study we did not detect any subject with potential CD. All antibody-positive subjects who underwent small bowel biopsy were found to have mucosal atrophy. One possible explanation for this finding is the adult age of our cohort. Since exposure to dietary gluten usually begins in early childhood, it is plausible that continuous exposure to gluten will trigger the histologic changes of CD in an accumulating number of subjects until adulthood. Previous studies have demonstrated that children with positive serologic testing and morphologically normal mucosa will become symptomatic at an annual rate of 2.8% [19].

Since selective IgA deficiency is 10–15 times more common in patients with CD than in the general population (1.7%–3%) [20], we elected to measure IgA serum levels in all screened patients. We found a 0.4% prevalence of IgA deficiency; however, all these patients tested negative for IgG anti-tTG. The absence of patients with CD among IgA-deficient subjects is probably related to the small size of the screened cohort.

Theoretically, there are many factors favoring mass screening for CD – a common disorder for which there is an effective and available treatment that leads to symptomatic relief and also prevents the complications of the disease. The crucial question is whether population-based screening should be considered outside of research programs. Most of the CD patients diagnosed by screening are asymptomatic. In our young adult population 88.9% of serologically positive subjects were clinically silent. Nonetheless, the question whether treatment in fact benefits clinically silent CD should be thoroughly assessed. Undetected CD increases the risk of complications, some of them life-threatening, like intestinal lymphoma [21]. On the other hand, the lifelong need to follow a strict gluten-free diet may be burdensome, especially when the patient is asymptomatic [22]. The natural history of undiagnosed CD remains unclear. Published studies have

been limited to patients who have received a clinical diagnosis, an approach that ultimately leads to a biased estimate of the risks [23]. The positive predictive value of serologic tests for CD (despite their high sensitivity) decreases when they are used in the general population rather than in groups at increased risk [24]. Further studies of the outcome of asymptomatic CD, including cost-effectiveness evaluations, are needed before population-based screening studies can be recommended.

In summary, we found that CD is highly prevalent in the young adult population in Israel. All subjects with a positive serology had duodenal mucosal atrophy, and therefore a gluten-free diet was indicated in all cases that were diagnosed by screening. Serologic screening for CD is a reliable and simple method for diagnosing this disease before symptoms or other complications develop.

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Capsule

Possible drug target for familial tumor genes

Inherited mutations in tumor suppressor genes cause an increased risk of developing familial cancer syndromes. Many of these familial tumor suppressor genes are also frequently mutated in somatic cancers. The tumor suppressor gene *NF2* is mutated in the familial cancer syndrome neurofibromatosis type 2, which causes multiple brain tumors such as schwannomas and meningiomas. *NF2* encodes the protein Merlin, which appears to link cell adhesion receptors at the cell surface to the actin cytoskeleton and is thus poised to inhibit mitogenic signaling downstream of integrins and adhesins. Li and collaborators have identified a very different function for Merlin, this time in the nucleus. Endogenous Merlin was observed in the nucleus of multiple cell types by virtue of its binding to an E3 ubiquitin ligase, CRL4DCAF1. The binding of CRL4DCAF1 to Merlin inhibited the ubiquitin ligase activity and suppressed cell proliferation. Tumor-derived mutations in *NF2* prevented Merlin from inhibiting CRL4DCAF1 activity, and CRL4DCAF1 was required for the malignant properties of primary human tumor cells derived from *NF2* patients, thus providing a possible drug target.

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Eitan Israeli

Preoperative Staging Using Transrectal Ultrasound in High and Low Rectal Cancer

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ABSTRACT: **Background:** An accurate preoperative definition of tumor and lymph node status is needed for reaching the correct decision regarding rectal cancer treatment. Transrectal ultrasonography is the most commonly used diagnostic modality for the local staging of rectal cancer.

Objectives: To determine the accuracy of TRUS in the staging of rectal cancer.

Methods: We conducted a retrospective study on 95 patients evaluated by TRUS. The rectum was subdivided into two parts (lower and upper).

Results: Sixty patients underwent radical surgery. Of these, 34 received no preoperative chemo-irradiation owing to μT1, μT2 tumor or the patient's choice (neo-adjuvant treatment was suggested to patients with adenocarcinoma that proved to be μT3). The overall accuracy rate was 80% for T stage. Overstaging was found in 13.3% and understaging in 6.7%.The N-stage was correctly assessed in 70%. The overall accuracy rate for tumors was 73.9% in the lower part and 90.9% in the upper. A trend towards a lower accuracy rate for low-lying tumors compared to high-located rectal tumors was found ($P = 0.532$), which did not reach statistical significance.

Conclusions: TRUS gave better results for T1 and T3 stage rectal tumors but was inaccurate for stage T2, indicating the possible need for local excision in order to base the final treatment for T2 tumors on pathologic staging.

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KEY WORDS: rectum, cancer, ultrasound, staging, histopathology

Rectal cancer is a common cause of death in Europe and the United States [1,2]. Prognosis is directly related to the tumor stage and the presence of lymph node metastases. Current treatment protocols for rectal cancer involve sphincter-saving surgery, transanal surgery, and neoadjuvant chemo-irradiation therapy, or a combination of more than one modality in addition to the longstanding traditional abdominoperineal resection. In order to make the right decision regarding rectal cancer treatment, an accurate preoperative definition of the tumor status (T stage) and lymph node status (N stage) is needed. The extent of tumor spread is generally evaluated by digital examination, transrectal ultrasonography, computed tomography and magnetic resonance imaging [3].

During the last decade TRUS became the most commonly used diagnostic modality for the local staging of rectal cancer. The accuracy rates of TRUS in assessing the depth of rectal wall invasion have ranged from 80% to 95% [1-6]. When assessing lymph nodes, TRUS has demonstrated an accuracy of approximately 58–83% [2-6]. The aims of the present study were to determine the accuracy of TRUS in the staging of rectal cancer compared with histopathologic examination, and to evaluate whether tumor site (in terms of distance from the anal verge) has an influence on the reliability of TRUS.

PATIENTS AND METHODS

In a retrospective study, data were collected on all patients with rectal cancer treated at Assaf Harofeh Medical Center in central Israel between July 2003 and September 2007. Ninety-five patients had a proven biopsy of rectal adenocarcinoma. After a rectal biopsy all 95 patients underwent TRUS using an ultrasound scanner, type Falcon 2101 (B&K Medical, Denmark) with a 10 MHz frequency probe.

All patients were evaluated by colonoscopy, CT of the chest, abdomen and pelvis, chest X-rays and a blood test for tumor markers. The distance of the tumor from the anal verge was measured by rigid rectoscopy. All TRUS examinations were performed by a single investigator (Y.Z.) using Lloyd-Davis stirrups.

During TRUS, rectal wall penetration was assessed using the modification of the TNM classification, based on a five-layer rectal wall model, proposed by Hildebrandt and Feifel [7]. Pathologic lymph nodes were defined as circular or slightly oval-shaped structures, with an echogenicity similar to the tumor, as proposed by Beynon et al. [8]. The surgical specimens were sent for histopathologic examination and the staging was classified according to the pTNM classification. The ultrasound staging was compared with the histopathologic staging of the resected specimen. Furthermore, to establish the tumor site, the rectum was subdivided into two sections – the lower part (0–5 cm from the anal verge) and the upper part (≥ 6 cm). If the tumor was not confined to one level only, the lower point of

TRUS = transrectal ultrasonography

the lesion was determined as the point of reference. The overall accuracy rates of the two levels were analyzed.

Statistical analysis was performed at Tel Aviv University's Department of Statistics, using the Measure of Agreement-Kappa test and Student's *t*-test; $P < 0.05$ was considered statistically significant.

RESULTS

Of the 95 patients examined by TRUS (42 males and 53 females, median age 66 years, range 33–92 years), 60 patients underwent radical surgery (anterior/low anterior resection in 35, transanal local excision in 15, abdominoperineal resection in 9, and total proctocolectomy with J-pouch in 1). Of the 60 patients, 34 did not undergo preoperative chemo-irradiation therapy because of their disease stage: μT1 in 14 patients, μT2 in 11 patients, and 9 patients with stage μT3 who chose not to have the treatment (neo-adjuvant treatment was suggested to patients with μT3 adenocarcinoma). The remaining 26 patients were treated with preoperative CRT. In 11 of these patients, TRUS was performed not only before but also after completion of CRT. The other 15 patients underwent TRUS only before CRT was given.

For the purposes of our analysis, the patients were divided into two groups: without CRT (group A, 34 patients) and TRUS after CRT (Group B, 11 patients). Patients who underwent TRUS only before CRT were excluded from the study since the purpose of our study was to evaluate the accuracy rate of TRUS.

Evaluating the depth of tumor invasion revealed an overall accuracy rate in both groups of 80% (36 of 45 patients) ($\kappa = 0.583$, $P < 0.01$). TRUS examination correctly staged 13 of 16 patients with T1 tumors (81.2%), 7 of 11 patients with T2 tumors (63.6%), and 16 of 17 patients with T3 tumors (94.1%). No correlation was found comparing TRUS and histopathologic findings in one patient with a T4 tumor. Using TRUS, overstaging was found in 6 of the 45 patients (13.3%) and understaging in 3 (6.7%).

The lymph node status was correctly assessed in 21 of 30 patients (15 of 45 patients underwent transanal local excision), an accuracy rate of 70% ($\kappa = 0.482$, $P < 0.01$) [Table 1]. Positive predictive value was 55.6%, negative predictive value 84.2%, specificity 80% and sensitivity 62.5%.

In the group of patients without CRT (group A), the overall accuracy rate of the depth of tumor invasion was 76.5% (26 of 34 patients) ($\kappa = 0.532$, $P < 0.01$). TRUS correctly staged 12 of 15 patients with T1 tumors (80%), 6 of 10 patients with T2 tumors (60%), and all 9 patients with T3 tumors (100%) [Table 1]. Overstaging was found in 5 of the 34 patients (14.7%) and understaging in 2 (5.9%).

The lymph node status was correctly assessed in 78.9% (15 of 19 patients) [Table 1]. Overstaging was found in 2 of the 19 patients (10.5%) and understaging in 2 (10.5%). In the group of patients in whom TRUS was performed before and after CRT (group B), the overall accuracy rate of the depth of tumor invasion was 90.9% (10 of 11 patients) ($\kappa = 0.750$, $P < 0.01$). TRUS correctly staged the one patient with a T1 tumor (100%), the one patient with a T2 tumor (100%), and seven of eight patients with a T3 tumor (87.5%). No correlation was found when comparing TRUS and histopathologic findings in one patient with a T4 tumor [Table 1]. Overstaging was found in 1 of the 11 patients (9.1%) and understaging in 1 (9.1%).

The lymph node status was correctly assessed in 54.5% (6 of 11 patients) [Table 1]. Overstaging was found in 3 of the 11 patients (27.3%) and understaging in 2 (18.2%). In this group, the rate of tumor downstaging after CRT with respect to the depth of tumor invasion was found in 2 of the 11 patients (18.2%). In one patient, TRUS showed a T3 tumor before CRT and a T1 tumor after CRT, and in another patient a T3 tumor before CRT and a T2 tumor after CRT. In these two patients a correlation was found between TRUS that was performed after CRT and the histopathologic findings. Tumor downstaging rate after CRT with respect to lymph node metastases was found in 4 of the 11 patients (36.4%).

Of the 45 tumors (groups A and B), 23 (51.1%) were located in the distal part and 22 (48.9%) in the proximal part of the rectum. Overall accuracy for tumors situated in the distal part of the rectum was 73.9% (17 of 23 patients) and 90.9% (20 of 22 patients) for tumors in the proximal part of the rectum [Table 2]. Although there was a trend toward a lower accuracy rate for low-lying tumors, this did not reach statistical significance ($P = 0.532$).

Table 1. Comparison of transrectal ultrasonography versus pathologic findings

	T1 tumor n (%)	T2 tumor n (%)	T3 tumor n (%)	T4 tumor n (%)	Overall n (%)	N stage n (%)
Group A: Without CRT	12/15 (80)	6/10 (60)	9/9 (100)	–	26/34 (76.5)	15/19 (78.9)
Group B: Post-CRT	1/1 (100)	1/1 (100)	7/8 (87.5)	0/1	10/11 (90.9)	6/11 (54.5)
Group A + B	13/16 (81.2)	7/11 (63.6)	16/17 (94.1)	0/1	36/45 (80)	21/30 (70)

CRT = chemo-irradiation, N stage = lymph node status

Table 2. Accuracy of transrectal ultrasonography according to tumor site in patients with pre- and post-CRT TRUS

Lower part (0–5 cm) n (%)	Upper part (≥ 6 cm) n (%)
17/23 (73.9)	20/22 (90.9)

DISCUSSION

To select the optimal treatment modality for patients with rectal cancer, accurate preoperative staging is necessary since it will

CRT = chemo-irradiation therapy

benefit the patients in terms of cure and quality of life. Depth of rectal wall tumor invasion and pararectal lymph node involvement have become important parameters when determining the type of treatment. An early small rectal cancer confined to the mucosa and submucosa can be excised locally by the transanal approach [9]; preoperative CRT is recommended for advanced rectal cancer for tumor downstaging, which may enable sphincter-saving procedures. The accuracy of TRUS in the evaluation of the depth of tumor invasion and regional lymph node involvement was found to be superior to or equivalent to that of digital examination, computed tomography and magnetic resonance imaging [3-6]. The accuracy rates of TRUS in determining the depth of tumor invasion and regional lymph node involvement have been reported to be 80–95% and 58–83%, respectively [1-6]. CT accuracy was found to range from 53% to 94% for depth of penetration and from 54% to 70% for lymph node metastases. MRI accuracy ranged from 66% to 92% for depth of penetration and from 60% to 90% for lymph node metastases [10]. In our study, the overall accuracy rate in determining the depth of tumor invasion and regional lymph node involvement was 80% and 70%, respectively. Overstaging was found in 13.3% of the 45 patients, and understaging in 6.7%. Thus, our results are comparable to those reported in the literature.

There is some controversy regarding the interpretation of the images obtained from TRUS. Our experience suggests that the accuracy of TRUS in assessing the depth of rectal wall invasion varies with tumor stage. It is more accurate for T1 (81.2%) and advanced tumors that penetrate the perirectal fat (94.1% accuracy rate for T3 tumors).

The endosonographic diagnosis of T2 tumors, which was only 63.6% in our series, was worse than previously reported but better than that reported (41% in T2) by Sailer and co-authors [11]. The low accuracy rate of TRUS in the diagnosis of T2 tumors emphasizes the need to base the final treatment, after transanal local excision, on the pathologic and not the ultrasound staging [12,13]. It is also difficult to diagnose a T4 lesion because of the short focal length of the transducer.

The high rate of tumor overstaging (13.3% in our study) was mostly due to tissue edema or an inflammatory reaction that cannot be easily differentiated from the tumor by TRUS. Understaging of the tumor was probably due to microscopic invasion, which cannot be detected by TRUS [14,15].

Whether tumor location, in terms of rectal level, has an impact on the endosonographic assessment of wall invasion is not yet settled. Sentovich and collaborators [16] reported a significantly better result for tumors within 6 cm of the anal verge. This is in contradiction to the study conducted by Herzog et al. [5] who found a significantly poorer accuracy rate for tumors of the distal third.

Our experience suggests that the accuracy rate of TRUS in assessing the depth of rectal wall invasion is higher for proximal than distal rectal tumors, but this is not statistically significant. A possible reason for the lower accuracy rate of tumor staging in the lower part of the rectum is the difficulty in reaching all sites of the ampulla recti with a rigid probe. Furthermore, the typical endosonographic five-layer structure of the rectal wall, as described by Hildebrand and Feifel [11], is somewhat less well defined at the level just above the anal canal.

In conclusion, the accuracy rate of TRUS examination in determining the depth of tumor wall penetration and regional lymph node involvement is high. TRUS gave better results for T1 and T3 rectal tumors and was relatively inaccurate for stage T2. The low accuracy rate of TRUS in the diagnosis of T2 tumors emphasizes the need to base the final treatment, after local excision, on the pathologic and not the ultrasound staging.

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Characterization and Clinical Outcomes of Drug-Eluting In-Stent Restenosis

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ABSTRACT: **Background:** The best therapeutic alternative for patients suffering from in-stent restenosis after drug-eluting stent implantation remains to be elucidated.

Objective: To characterize the pattern, treatment and outcomes of DES-related in-stent restenosis in patients treated at our institution.

Methods: We determined the incidence and major adverse clinical events in 71 consecutive patients with DES failure among 2473 patients who were treated with 2548 drug-eluting stents between 2004 and 2007. We analyzed the clinical data, procedural parameters and clinical outcomes.

Results: The type and number of stents implanted were as follows: Cypher (n=1808), Endeavor (421) and Taxus (319); of these, 53 (2.9%), 10 (2.4%), and 8 (2.5%) patients respectively presented with restenosis. The mean time to restenosis was 11.3 ± 9.9 months. Patients' mean age was 65 ± 11 years; 75% were male, and 68% had diabetes mellitus. Unstable angina was the clinical presentation in 52 (73%). At 6 months, 3 patients had developed myocardial infarction (4.2%), repeat restenosis at follow-up was diagnosed in 8 patients (11.3%), the overall major adverse clinical events rate was 18.3% (13 patients), and 2 patients died (2.8%).

Conclusions: Drug-eluting stent-related restenosis is relatively infrequent but remains a clinical challenge. It occurs more frequently in complex lesion subsets, but the overall intermediate-term prognosis is tolerable.

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KEY WORDS: in-stent restenosis, drug-eluting stent

Drug-eluting stents were designed to prevent in-stent restenosis. However, the optimal management of DES-related restenosis has yet to be defined. The results of multiple studies suggest that the DES implant is the treatment of choice for patients with ISR after bare-metal stent implantation [1-5]. Although the use of DES has significantly decreased restenosis, there is little information on the long-term results of in-stent restenosis following DES implantation. Thus, the challenge remains to identify the best therapeutic options for patients presenting with ISR after DES implantation.

Our study investigated the clinical characteristics and pattern of restenosis, including its association with different DES types, the outcome at 6 months relative to the pattern of restenosis, and the general outcome for coronary patients.

DES = drug-eluting stent
ISR = in-stent restenosis

PATIENTS AND METHODS

In our institution, the rate of DES is ~40% and is regulated by restricted indications such as proximal to mid-left anterior descending or proximal left circumflex lesions, diffuse coronary stenosis, in-stent restenosis and total coronary artery occlusion. Between January 2004 and February 2007, we identified 2473 consecutive patients who underwent 2548 DES implantations (71% Cypher® eluting sirolimus drug, 17% Endeavor® eluting zotarolimus drug, and 12% Taxus® eluting paclitaxel drug). Of these, we identified 79 consecutive patients with DES failure: 71 who experienced DES restenosis and 8 who had DES-related thrombosis.

In-stent restenosis was defined as a luminal stenosis of at least 50% located within the stent or within 5 mm of the stent edges. The ISR pattern was classified according to the Mehran system [6] as follows:

- Focal ISR lesions were defined as ≤ 10 mm of length, in the body and/or the edges of the stents
- Diffuse ISR lesions were diffuse lesions ≥ 10 mm in length occurring intrastent, and they were proliferative, extending beyond the stent margins
- Occlusive lesions.

The clopidogrel loading dose was 300–600 mg. A clopidogrel loading regimen was administered prior to interventions (e.g., 6–24 hours) in all acute coronary syndrome patients and immediately following the procedure in other cases. The duration of clopidogrel therapy was at least 3–12 months for those with a Cypher stent, 6–12 months for those with Taxus stents, and 3–12 months for patients with an Endeavor stent.

Renal failure was defined as a creatinine level > 1.4 g/dl. Major adverse cardiac events beyond the index DES failure event were defined as death, myocardial infarction (Q or

non-Q according to chest pain, electrographic changes, and positive troponin), target vessel revascularization, and/or need for unplanned bypass surgery.

The registry includes detailed demographic, clinical, angiographic and procedural data on the 71 patients who experienced a DES-related restenosis event. Immediate and in-hospital events were recorded, and each patient was surveyed by telephone or at the outpatient clinic with a standardized questionnaire at 30 days, 6 months, and 1 year of follow-up. Survival status at follow-up was determined by the national registries. Repeat revascularization procedures and episodes of acute myocardial infarction were prospectively collected in the hospital database. For patients admitted in the acute phase to peripheral hospitals, the diagnosis of myocardial infarction was confirmed by source documentation obtained from the referring physician.

ANGIOGRAPHIC ANALYSIS

Angiographic films were reviewed at our angiographic core laboratory using the MDView™ Quantitative Angiographic System (Medcon-McKesson, Telemedicine Technology, Tel Aviv, Israel). Analysis was performed by an experienced cardiologist who was unaware of the clinical outcome. Standard morphologic criteria were used for the identification of lesion location, lumen diameters, length, and existence of thrombus. Percent diameter stenosis was determined using standardized calibrated methods before and after interventions, as was TIMI flow grade (0 to 3) before and at the completion of the intervention. We also performed subanalyses on groups according to the ISR pattern and focal restenosis in the Cypher stent (the sirolimus-eluting stent) compared to the Endeavor (zotarolimus-eluting) stent.

STATISTICAL ANALYSIS

Continuous variables are presented as mean ± standard deviation. Statistical analysis was performed using STATISTICA software (StatSoft, Inc. Tulsa, OK, USA), and $P < 0.05$ was considered significant for all analyses.

RESULTS

We obtained baseline demographic and clinical data of 71 consecutive patients who experienced DES-related restenosis events [Table 1]. Patients who developed ISR were more likely to have diabetes mellitus (68%), and 20% of these were insulin-dependent. A total of 44% of patients had a prior history of myocardial infarction and 41% had previous bypass grafts (41%); 80% of the patients had multivessel coronary artery disease. Unstable angina was more likely to be the presentation in this group of patients (73%). Mean time to restenosis was 11.3 ± 9.9 months.

Angiographic data for quantitative analysis are also given in Table 1. Lesion length and stent diameter were on average 3.0 ± 0.4 and 12.8 ± 9.1 mm, respectively.

Table 1. Clinical and coronary angiography data

Patients	**N=71**
Age (yrs)	65 ± 11
Male	53 (75%)
Hypertension	57 (80%)
Dyslipidemia	60 (85%)
Smoker	12 (17%)
Diabetes	48 (68%)
Insulin-dependent	10/48 (20%)
Renal failure	13 (18%)
Previous infarction	31 (44%)
Previous bypass	29 (41%)
Stroke	7 (10%)
Ejection fraction (%)	47 ± 10
Multivessel disease	57 (80%)
Angiography data	
Pre-procedure	
Reference diameter (mm)	3.0 ± 0.4
Minimal diameter (mm)	0.47 ± 0.58
Diameter stenosis (%)	85 ± 18
Lesion length (mm)	12.8±9
Post-procedure	
Reference diameter (mm)	3.2 ± 0.6
Minimal diameter (mm)	2.6 ± 1.0
Diameter stenosis (%)	22 ± 30
Stent diameter (mm)	3.0 ± 0.4
Stent length (mm)	23.3 ± 7.6

CLINICAL OUTCOME AND RESTENOSIS PATTERN

Data were available for all patients at 6 months. The overall rates of death and of myocardial infarction were 2.8% and 4.2%, respectively. Recurrent restenosis occurred in 8 patients (11.8%) and the overall MACE rate was 18.3% (13 patients).

Focal restenosis, according to the Mehran classification [6], constituted the majority of lesions. Restenosis treatment was at the discretion of the clinician, and the re-stenting rate of the lesion was 60% whether it was focal or diffuse. All patients who received an additional stent were treated using another DES. In other words, no bare metal stents were used to treat DES-related in-stent restenosis. Focal ISR was found in 71%, diffuse ISR in 22%, and 7% presented with stent occlusion. Re-stenting was more often performed for focal and diffuse patterns (68% and 53%, respectively) followed by balloon angioplasty alone (26% and 27%, respectively), as compared to bypass surgery or medical therapy in the occlu-

MACE = major adverse clinical events

Table 2. Six month outcome according to in-stent restenosis type

	Focal (N=50)	Diffuse (N=12)	Occlusion (N=5)	*P value*
Death	0	1 (8.3%)	0	0.2
Myocardial infarction	2 (4.2%)	1 (8.3%)	0	0.4
Repeat revascularization	5 (10.6%)	4 (33%)	0	0.08
Target vessel revascularization	5 (10.6%)	3 (25%)	0	0.2
Major adverse cardiac events	7 (14.6%)	5 (42%)	0	0.05

sive pattern. Six month outcomes according to restenosis type were available in 67 patients. The incidence of recurrent coronary intervention increased in the diffuse restenosis type compared to the focal group, as did MACE, with frequencies of 42% versus 14.6% respectively ($P = 0.05$) [Table 2]. There were no increments in mortality or myocardial infarction among the subgroups.

RESTENOSIS ACCORDING TO STENT TYPE

A comparison of Cypher and Endeavor Sprint stents (the "limus" eluting stents) showed Cypher failure in 53 patients (age 64 ± 11 years, 79% male) with 57 lesions who presented with acute coronary syndrome. Ten patients had Endeavor failure (age 72 ± 8.7 years, 80% male). Restenotic patients were often characterized as high risk in both groups, with diabetes (73.5% vs. 80%), hypertension (83% vs. 100%), and dyslipidemia (89% vs. 90%). Cypher stent lengths were 24 ± 8 vs. 19 ± 6 mm for the Endeavor stents ($P = 0.07$), with stent diameters averaging 3.0 ± 0.4 mm (Cypher) vs. 3.2 ± 0.5 mm (Endeavor) ($P = 0.1$). Mean time to DES failure was 12.5 ± 10.6 months for Cypher and 5.2 ± 2.7 months for Endeavor ($P < 0.05$). The vast majority of restenotic lesions (71%) were focal in the Cypher group and diffuse (80%) in the Endeavor group ($P = 0.004$). Accordingly, the incidence of diffuse restenosis was significantly higher in the Endeavor compared to the Cypher stents (12% vs. 80%, $P < 0.0002$). At the 6 month follow-up, the overall MACE (death, myocardial infarction, target vessel revascularization) was 11.3% in the Cypher group and 50% in the Endeavor group ($P = 0.01$). Only four patients in our series presented with Taxus-related restenosis. All four were alive at 6 months follow-up.

DISCUSSION

DES implants have greatly decreased the incidence of restenosis and the need for target lesion revascularization; however, restenosis remains a significant problem because of the increased number of complex disease interventions. Most studies have identified angiographic restenosis rates of 3% to 12% for in-stent or in-segment restenosis, depending on the patient's characteristics and lesion complexity [7]. However, the clinical restenosis rate following DES implantation is even lower.

The present study identified a 2.9% rate of clinical restenosis after DES implantation in the "all comers" group of patients treated at our hospital. The angiographic morphology of restenosis following DES implantation showed focal restenosis as the predominant pattern, especially with the Cypher stent. This pattern of DES-related restenosis, which is easier to treat, has already been described in several studies [8,9]. Reporting their experience with post-DES restenosis, Lee et al. [10] stated that focal ISR occurred in 62% of lesions, while diffuse or proliferative ISR was present in 29% and total occlusion in 9%. Hong and associates [11] reported late need of repeat revascularization, beyond 6 or even 9 months, which was performed in 1.8% of native lesions that were patent on the 6 month follow-up angiogram. Two-thirds of late restenotic lesions had a focal angiographic restenotic pattern [11]. Our data showed that a focal restenotic pattern was the most representative in the Cypher group (79%), while in the Endeavor stent group the pattern was more diffuse. We also described the treatment type when specifically analyzing the subgroup of focal post DES-related restenosis.

A meta-analysis of six randomized trials has indicated a modest benefit with sirolimus-eluting stents compared to paclitaxel-eluting stents [12]. Taxus stents entered the Israeli market after the Cypher and Endeavor stents, and in the present study we compared Cypher and Endeavor, both of which are "limus"-based stents – to detect a restenotic pattern. Based on our experience and compared to the Cypher stent, the Endeavor DES failure showed a more diffuse restenotic pattern, a shorter time to restenosis, and worse overall intermediate-term clinical outcome. Miyazawa et al. [13] in their IVUS study (ENDEAVOR III), compared the vascular response of Endeavor and Cypher stents at baseline and after 8 months and showed larger IVUS-detectable neointimal coverage over stent surface at 8 months. Kandazari et al. [14] found that compared with Cypher, treatment with Endeavor is associated with significantly higher in-segment late lumen loss and binary restenosis at the 8 month angiographic follow-up. This has not been translated into a major clinical adverse outcome.

The preferred treatment approach for DES restenosis is not yet established, but the options include repeat intervention with another DES, use of the same type of stent or use of a stent with a different antiproliferative agent, revascularization with balloon angioplasty/cutting balloon, or bypass surgery. More recently, the use of drug-eluting balloons (rather than drug-eluting stents) may become a viable treatment option for DES failure due to restenosis events [15]. The TAXUS ARRIVE Registry included more than 2400 patients and more than 3000 lesions treated with the Taxus stent. Among those treated with repeat stenting, 5% received bare metal

stents and 58% were treated with DES [16]. Of those treated with DES, 77% were retreated with Taxus stents and 23% treatments involved another DES, mainly Cypher. In our group, treatment type was at the discretion of the clinician, and we found that 69% of focal ISR were re-stented while 27% were treated with balloon angioplasty alone. Patient outcome according to treatment showed a tendency to a better outcome in the balloon angioplasty group but it did not reach statistical significance. Of 57 lesions with Cypher-related stent restenosis, 27 (53%) were treated with the same stent. Some operators preferred to switch stent types in this setting (e.g., Taxus for Cypher restenosis and vice versa), but there were no data to support this approach.

Solinas and team [17] described their experience with repeated DES treatment for DES restenosis, reporting that treatment with either repeated DES implantation or balloon angioplasty for DES-ISR was safe and associated with low overall rates of target-lesion revascularization and major adverse cardiac events at 1 year. They also found that implantation of a different DES type (the "switch" strategy) may result in more favorable outcomes compared to the same DES (no-switch strategy) that had failed originally. Regarding the overall outcome of patients with DES restenosis, Garg et al. [18] found that the repeat revascularization rate for patients treated with a different DES was 14.5%, and 16.7% for the same DES; this difference was not significant. Other studies showed similar data after DES failure regardless of the treatment modality [19,20].

Our study has several limitations: it involved a small group of patients/lesions and was a retrospective analysis. In addition, the treatment groups were not randomly assigned, and no angiographic or ultrasonic follow-up data were obtained.

In summary, according to our experience, DES-related restenosis is relatively infrequent but remains a clinical challenge. It occurs more frequently in complex cases and lesion subsets, but the overall intermediate-term prognosis following repeat percutaneous treatment is acceptable. Further studies regarding the best treatment strategies for focal or diffuse lesions are needed. Treatment options could include using a balloon or cutting balloon and/or drug-eluting balloon or using another drug-eluting stent. Finally, it should be remembered that referring the patient to a coronary bypass surgery is another option for recurrent DES failure.

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"A friend is one before whom I may think aloud"

Ralph Waldo Emerson (1803-1882), American essayist, philosopher, and poet

Age-Related Immunoglobulin G Seroprevalence of Human Parvovirus B-19 in Israeli Children

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ABSTRACT: **Background:** Human parvovirus B19 is a global and common infectious pathogen in humans, particularly in children.
Objectives: To assess the immunoglobulin G seroprevalence of B19 in children in Israel.
Methods: Overall, 128 previously healthy children (1.5–17 years old) hospitalized for various diseases other than acute human parvovirus B19 infection were assessed for IgG to the virus by enzyme-linked immunosorbent assay.
Results: The IgG seroprevalence increased from 22% in children aged 1.5–9 years to 52% in older children (P = 0.001).
Conclusions: Our data suggest that most acute parvovirus B19 infections in Israel occur in the early school years, and that by 18 years of age 50% of Israeli children have been infected by the virus.

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KEY WORDS: parvovirus B19, immunoglobulin G, seroprevalence, child

Human parvovirus B19, a member of the family Parvoviridae, is a global and common infectious pathogen in humans, primarily in children [1]. In previously healthy children, symptomatic B19 infection has been associated with several clinical syndromes, such as various exanthema diseases, acute arthropathies, and acute hematologic manifestations including transient aplastic crisis or pancytopenia. Additionally, B19 infection of patients with chronic hemolytic diseases may be associated with severe aplastic crisis. In many children, the infection may be either asymptomatic or non-typical [1-3], but they may transmit the infection to susceptible children and adults including pregnant women. Acute B19 infection occurs in 1–5% women during pregnancy, and the prevalence increases to 3–20% during epidemics. Although transplacental fetus infection occurs in about 30–50% of acutely infected pregnant women, most fetuses develop normally. However, maternal infection during the first 20 weeks of pregnancy may be associated with severe anemia, non-immune hydrops fetalis and fetal loss in up to 9% if undiagnosed and untreated [1,10]. Data on B19 epidemiology in Israeli children are lacking.

The aims of the current study were to assess the IgG seroprevalence of B19 among healthy children in Israel, and evaluate the possible susceptibility for B19 infection among women of childbearing age.

PATIENTS AND METHODS

This study is a continuum of a recently published study evaluating the role of B19 infection in Israeli children aged 1.5–17 years hospitalized with various clinical syndromes compatible with acute B19 infection. The study was prospective and ran from 1 October 2002 to 30 August 2004 in three pediatric departments in Israel – HaEmek Medical Center in Afula, Sieff Medical Center in Safed, and Shaare Zedek Medical Center in Jerusalem. Institutional review boards in these hospitals approved the study and informed consent was obtained from parents before inclusion.

The study group comprised 167 children, whose mean age was 5.5 ± 4.6 (range 0.5–17) years. Each enrolled child had a serum specimen tested for the presence of parvovirus B19 DNA by real-time polymerase chain reaction and for the presence of anti-parvovirus B19 IgM and IgG antibodies, tested by enzyme-linked immunosorbent assay [3,4]. By definition, a hospitalized child with compatible symptoms who had either B19 DNA by real-time PCR, or anti-parvovirus IgM, was considered as having acute B19 disease.

Of the 167 hospitalized children (age 1–17 years) in the study, 128 were found not to have acute B19 disease. Analysis of the IgG seroprevalence in this latter group constitutes the current study.

Data analysis was performed using the SPSS statistical

IgG = immunoglobulin G
B19 = human parvovirus B19

PCA = polymerase chain reaction

Table 1. Age-related parvovirus IgG seroprevalence in Israeli children

Age (yrs)	1.5–5	6–9	10–15	16–17
Overall	54	43	23	8
IgG positive PCR and IgM negative	11	10	12	4
%	20.4%	23.2%	52%	50%

package. Prevalence rates of B19 IgG seroprevalence were compared by chi-square test. *P* values were two-sided, with a significance level of $P < 0.05$.

RESULTS

The 128 children who fulfilled the inclusion criteria of this study were equally distributed between males and females. IgG seroprevalence in these children is shown in Table 1. The overall B19 IgG seroprevalence increased from 21.6% (21/97) in children 1.5–9 years old to 51.6% (16/31) in older children ($P = 0.001$ by chi-square test). No significant differences in seroprevalence rates were documented among age groups of children between boys and girls.

DISCUSSION

The present study, the first in Israel to assess age-related B19 IgG seroprevalence in children, shows that by 18 years of age about 50% of Israeli children have been infected by B19. Our results are concordant with recently published data from other countries, mostly European, showing that the percentage of people with measurable levels of B19-specific IgG increases with age from 5–40% at age 1–9 years to 40–63% at age 10–18 years, with most individuals becoming infected during their school years [Table 2]. Vyse et al. [8] showed that B19-specific IgG prevalence rose non-linearly with age from 21% in those aged 1–4 years to > 75% in adults aged ≥ 45 years. Force-of-infection estimates were similar to those made in 1991, the highest being in those aged < 15 years. There was no association between evidence of previous infection and gender or region. This phenomenon can be explained by the combination of two factors: namely, the mode of transmission of the virus in the community and the relatively high prevalence of the disease in childhood.

B19 is transmitted primarily through respiratory secretions and saliva. Therefore, in crowded environments such as daycare centers, kindergartens and schools, transmission of the virus from infected to non-infected individuals is probable [1].

The most typical disease associated with B19 in children is erythema infectiosum, which is clinically easy to diagnose and peaks at age 7–9 years. However, acute B19 infection may also be sub-clinical or presents with other non-specific manifestations associated with the virus. Barash and co-authors [2] described the clinical presentation of 40 children hospitalized with acute B19 infection as diagnosed by the presence of B19 IgM. They found that prolonged, recurrent or intermittent fever appeared in two-thirds of the children of whom most were over 4 years of age, and it was accompanied by a rash in only a few. Other manifestations included arthropathy and unexplained non-responding anemia. In our prospective original study we detected acute B19 disease in 21 of 149 children (12.6%) of whom 10 presented with a variety of acute exanthema diseases (none had typical erythema infetiosum); 5 presented with acute arthropathy (all 5 had transient synovitis), 4 presented with transient pancytopenia or aplastic anemia, and 2 children presented with fever of > 1 week [3]. Tuckerman and collaborators [9] described an outbreak of erythema infectiosum in a village primary school, where the course of disease in 14 of 64 children and adults (22%) with serologically proven recent B19 infection was sub-clinical.

We are not aware of any study in Israel assessing B19 seroprevalence among adults. Studies conducted in Europe show a prevalence of 30–60% in adults and more than 85% in the geriatric population. As shown in Table 2, in countries with a B19 seroprevalence rate among children similar to that of Israel, 30–40% of women aged 18–19 years are susceptible to B19 infection. Parvovirus infection during pregnancy can cause severe fetal anemia as a result of fetal erythroid progenitor cell infection with a shortened half-life of erythrocytes, causing high output cardiac failure and subsequently non-immune hydrops fetalis [1,10]. If data in Israel are concordant with those of Europe, and assuming 0.6–3% primary infection during the first two trimesters of pregnancy and 150,000 pregnancies/year in Israel based on

Table 2. Results of recent published surveys of IgG B19 seroprevalence in various counties

Author [ref], yr	Year of survey	No. of children studied	Country	1–9 yrs	10–18 yrs	Susceptible at 18–19 yrs**
Kelly et al. [5], 2000	1992-8	202	Victoria, Australia	28%	51%	33%
Mossong et al. [7], 2008	1996	NA	England & Wales	22–33%	60–63%	38%
Mossong et al. [7], 2008	1997-8	NA	Finland	5–30%	30–50%	49%
Mossong et al. [7], 2008	2003-4	NA	Italy	10–40%	40–60%	40%
Mossong et al. [7], 2008	1995-2004	NA	Poland	20–40%	40–60%	40%
Vyse et al. [8], 2008	1997-8	426	Germany	40%	55%	33%
Current study	2004	128	Israel	22%	52%	NA

*Rounded numbers

** Susceptible to acute B19 infection

NA = not available,

available data, it is estimated that potentially about 900–4800 pregnant women might be infected with B19, resulting in up to 50% (450–2400) infected fetuses and a possible fetal loss of up to 9% (40–215) per year. It has been shown that early detection and diagnosis of the infection and fetal anemia by amniocentesis and/or cordocentesis leading to repeated transfusion of erythrocytes to the fetus may lower the mortality rate associated with non-immune hydrops fetalis from 50 to 18% [10]. Since data regarding B19 IgG seroprevalence among childbearing women in Israel are lacking, a prospective study assessing this issue as well as the prevalence of B19-associated non-immune hydrops fetalis is needed in order to plan a national preventive approach.

In conclusion, B19 infection is frequent in children in Israel and by 18 years of age about 50% of children have been infected, suggesting a relatively high susceptibility to B19 infection among young pregnant women.

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Capsule

Development of metabolic syndrome influenced by gut microbes

Obesity, now officially recognized as an epidemic in many developed nations, is a key component of "metabolic syndrome," an array of metabolic disturbances that increase an individual's risk of developing diabetes and heart disease. The rise in obesity rates has been largely attributed to the growing imbalance between food intake and energy expenditure, but recent provocative work has suggested a possible link between obesity and the composition of microbes residing within the gut. Vijay-Kumar and co-authors found that mutant mice deficient in a component of the innate immune system develop hallmark features of metabolic syndrome, accompanied by changes in gut microbiota. Notably, transfer of gut microbiota from the mutant mice to wild-type mice conferred several features of metabolic syndrome to the recipients. Thus, the development of metabolic syndrome may indeed be influenced by gut microbes that are regulated by the innate immune system.

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Eitan Israeli

Capsule

Generating mice with T cells specific to antigens from a variety of infectious diseases

T cell receptor (TCR) transgenic mice are one of the most useful and ubiquitous tools of the immunologist. This is because the majority of T cells that develop in these mice express T cell receptors with known antigen specificity, and thus the mice can be used to study antigen-specific immune responses. The downside of TCR transgenic mice is that they can be difficult and time consuming to generate and the antigen specificities of their T cells are often not physiologically relevant. Kirak and team describe the use of somatic cell nuclear transfer to create TCR transgenic mice with specificity for antigens known to be important in the immune response against the parasite *Toxoplasma gondii*. This method generates mice with greater ease and speed than conventional TCR transgenic mice and can be applied to generate mice with T cells specific to antigens from a variety of infectious diseases.

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Eitan Israel

The Endless Differential Diagnosis of Acute Obstructive Renal Failure: Unusual Challenges for the Sharp-Sighted Clinician

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ABSTRACT: Obstruction of urine outflow can result from mechanical blockade as well as from functional defects. In adults, urinary tract obstruction is due mainly to acquired defects, such as pelvic tumors, calculi, and urethral stricture. In childhood it is mostly due to congenital malformations. In this article we present two rare cases of acute obstructive renal failure that presented with hydronephrosis. These cases underline the wide range of causes that may lead to this clinical feature.

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KEY WORDS: obstructive renal failure, bladder metastasis, gastric signet-ring cell carcinoma, CHARGE syndrome, genitourinary abnormalities

Urinary flow obstruction can occur anywhere from the renal pelvis to the urethra. The development of renal insufficiency in patients without intrinsic renal disease requires bilateral obstruction (or unilateral obstruction with a single functioning kidney). We often encounter this clinical entity in patients with prostatic disease, which causes mechanical obstruction to the urinary outflow; however, there are numerous other causes such as calculi, blood clots, infections and tumors.

In this report we will elaborate on two rare causes of obstructive uropathy; the first is a rare case of a patient with gastric signet-ring cell adenocarcinoma who presented initially with hydronephrosis and acute renal failure as a result of metastases to the bladder. The second is the first reported case of a woman with CHARGE syndrome, a rare congenital syndrome that includes multiple congenital anomalies of the genitourinary system and other systems. She was diagnosed in adulthood after an episode of acute obstructive renal failure. These two cases represent the wide range in the differential diagnosis of obstructive uropathy, including pathologic conditions of all disciplines in medicine.

PATIENT DESCRIPTIONS

PATIENT 1

This 83 year old woman had a history of hypothyroidism, essential hypertension and ischemic heart disease. She was cured from left breast cancer 7 years previously. She was admitted because of acute renal failure, with an elevated creatinine level of 8 mg/dl. Sonography of the kidneys demonstrated severe left renal hydronephrosis. A nephrostome was inserted and the creatinine level decreased, stabilizing at 1.2 mg/dl. Gross hematuria later appeared. After withdrawing antiplatelet aggregation therapy and ensuring kidney function, the nephrostome was removed.

A computed tomography scan showed ascites and infiltration of the omentum. Retroperitoneal lymph nodes were observed, as well as uniform thickening of the bladder. Pelvic and abdominal organs, including the gastrointestinal wall, appeared normal. Gynecological examination and transvaginal ultrasound did not reveal any masses in the ovaries or uterus. Urinary bladder biopsy showed tissue infiltrated by a signet-ring cell adenocarcinoma, but no evidence of carcinoma *in situ* was found. Immunohistochemical study of the bladder biopsy was positive for cytokeratin 20 and negative for estrogen receptor [Figure 1]. Ascitic fluid and pleural effusion were examined: Periodic acid-Schiff stain of the cytologic material was positive for neoplastic cells, indicating possible gastrointestinal origin of the tumor.

Metastasis of gastric origin to the bladder was the first probable diagnosis. The final diagnosis was based on upper gastrointestinal endoscopy and biopsy that revealed primary signet-ring cell carcinoma of the stomach. The patient developed urinary tract infection and sepsis, with subsequent multiorgan failure. She succumbed to her disease 2 months following her initial admission.

PATIENT 2

The patient was a 46 year old Arab woman who had been diagnosed in childhood with total left eye and partial right eye blindness, bilateral coloboma, complete right ear deafness and anosmia. She was mentally retarded, and her height was 1.5 meters. She had bilateral facial paralysis. The gynecological history of the patient was unclear: she had occasional urinary bleeding, but had primary amenorrhea. She had never had a proper medical examination, including gynecological.

She presented with abdominal and right flank pain, nausea and vomiting. Creatinine level was 1.5 mg/dl. Ultrasonography of the kidneys revealed right hydronephrosis. An abdominal and pelvic CT scan showed a dysplastic, small, non-functioning left kidney, and a cystic pelvic mass that was interpreted as a bladder diverticulum containing a stone. A gynecological examination was not possible because of the absence of a vaginal orifice. A pelvic laparoscopy revealed pus in the pelvis and a right unicornate uterus, ovary and fallopian tube. The left ovary and fallopian tube were absent. A CT scan demonstrated malrotation of the right colon, without volvulus [Figure 2]. A right nephrostome was inserted, with a subsequent daily urine output of > 6 L. After several days of follow-up, both the urine output and creatinine level decreased gradually until the creatinine stabilized at normal ranges.

Ten days later, the patient was again hospitalized because of recurrent abdominal and right flank pain. Urine output was normal, and she did not urinate through the urethra. Both CT scan and rectal examination suggested hematocolpos. Urinalysis and cultures revealed urinary tract infection.

Treatment with fluids and antibiotics was initiated. The patient refused any further gynecological examination. Her medical condition was stable, and she was released. Based on her medical history and the present findings, the patient was discharged with a diagnosis of CHARGE syndrome.

Figure 1. Bladder biopsy: metastatic gastric signet-ring cells showing positive reaction for immunohistochemical stain with cytokeratin 20

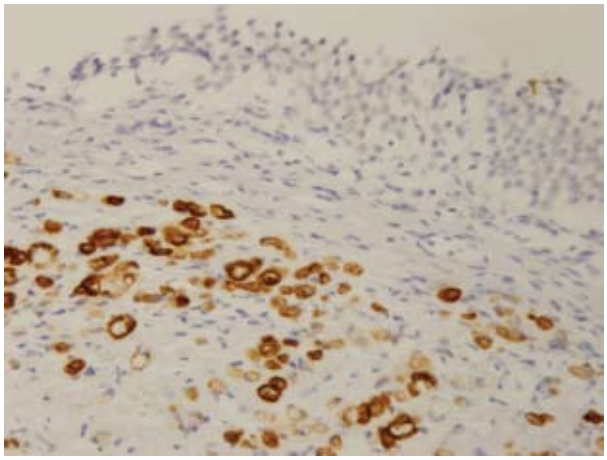
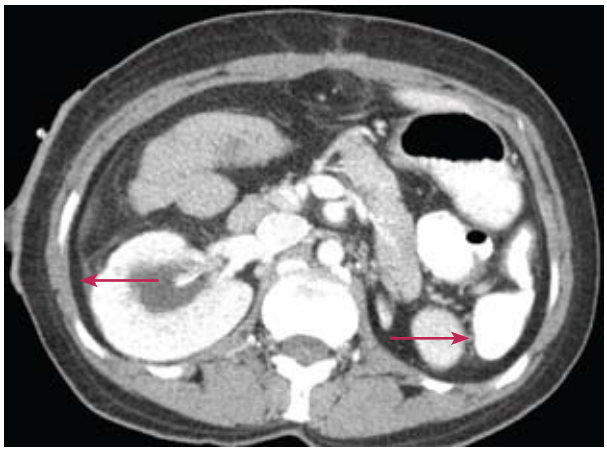


Figure 2. CT scan showing right hydronephrosis, nephrostome in the right kidney (red arrow) and malrotation of the right colon, without volvulus



DISCUSSION

Urinary tract obstruction accounts for fewer than 5% of cases of acute renal failure. It can be classified on the basis of location in the urinary tract, either in the upper urinary tract (above the ureterovesical junction) or in the lower urinary tract [1].

In the upper urinary tract an obstruction can result from intrinsic or extrinsic mechanical blockade. Extrinsic lesions that lead to obstructive uropathy originate from the vascular system, reproductive system, gastrointestinal tract and the retroperitoneum. In the lower urinary tract the lesions can originate from the meatus, urethra, prostate and bladder. In both our cases the presenting symptom was hydronephrosis. Hydronephrosis has been found in 3.5–3.8% of all postmortem examinations, with an equal male-female distribution. At autopsy, the frequency in children is 2% and is primarily due to congenital anomalies of the urinary tract. Obstruction related to renal calculi is three times more common in men than in women. Between the ages of 20 and 60 years, urinary obstruction is more common in women and is due to pregnancy and pelvic cancer. After the age of 60 most cases are seen in men and are related to prostatic hypertrophy or malignancy [2,3].

The first patient described here developed obstructive renal failure as a result of bladder metastases originating from gastric signet-ring cell adenocarcinoma. Secondary bladder neoplasms represent no more than 3% of all malignant tumors in surgical specimens, of which distant metastases from the stomach account for about 4% [4]. Adenocarcinoma of the bladder comprises fewer than 2% of all bladder tumors,

in most cases as a result of direct invasion from the surrounding organs [5,6].

Adenocarcinomas originating from different organs have a similar histologic appearance. Immunohistochemical staining profile may be helpful in determining the primary origin of the neoplasm. Breast and gastric cancer are the most common primary sites of signet-ring cell carcinoma. By combining the results of cytokeratin 20 and estrogen receptor staining, all metastases can be properly classified. The cytokeratin 20+/ER- pattern is characteristic of gastrointestinal tumors [6,7].

Only five cases of gastric signet-cell adenocarcinoma with metastases to the urinary bladder have been described in the literature: in four cases, the patients were already diagnosed with gastric signet-ring cell adenocarcinoma and underwent a gastrectomy prior to the uorologic presentation. In one of these four cases the disease recurred as hydronephrosis. The previously reported patients were between the ages of 52 and 59, whereas our patient was much older [4,5,8,9].

We present the sixth case of gastric signet-ring cell adenocarcinoma with metastases to the bladder. In contrast to the other cases, in our patient the initial presentation of the primary gastric tumor was urologic with left hydronephrosis and acute renal failure. Our second case was a woman with CHARGE syndrome that was diagnosed in adulthood after an episode of acute obstructive renal failure due to congenital genitourinary malformations.

The acronym CHARGE summarizes six cardinal features: **c**oloboma, **h**eart defect, **a**tresia of the choanae, **r**etarded growth and development, **g**enitourinary anomalies, and **e**ar anomalies/deafness. CHARGE syndrome is often associated with dysfunction of multiple cranial nerves, particularly the first (anosmia), the seventh (facial palsy) and the eighth (sensorineural hearing loss). The syndrome has an estimated incidence of 0.1–1.2:10,000. It affects both genders equally and occurs in many races. About 60% of patients have mutations in the *CHD7* gene [10,11].

CHARGE syndrome is one of several conditions characterized by multiple congenital anomalies that should be considered in a patient with both auricular and renal anomalies. Neurosensory hearing loss was found to be associated with renal agenesis, and its incidence among patients with chronic renal failure is considerably higher than in the general population. A close connection seems to exist between renal diseases and hearing disorders. According to Abbasi et al. [12] this link might be explained by the fact that similar proteins exist in both renal and ear tissues. When auricular anomalies are present, a careful assessment for accompanying dysmorphic features should be performed, and when present a renal ultrasound is mandatory [13,14].

According to Ragan and colleagues [15] there is a high incidence of genitourinary anomalies in CHARGE syndrome. They reviewed 32 patients who were diagnosed with CHARGE syndrome and found that 69% had genitourinary abnormalities, including atresia of uterus, unicornate uterus, cervix and vagina, and hypoplastic female labia and clitoris. Renal anomalies have also been reported, including solitary kidney, hydronephrosis, renal hypoplasia and nephrolithiasis. Therefore, patients with this condition should undergo a careful genitourinary evaluation, including renal and bladder ultrasound, and voiding cystourethrography screening [10,11,15].

In this article we presented two rare cases of obstructive renal failure apparently caused by a common simple mechanism: compression of the lower urinary tract. Based on these two cases we conclude that a high index of suspicion is needed to enable early and accurate diagnosis and concomitant treatment and follow-up of patients with obstructive renal failure.

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A Pertussis Outbreak among Daycare Children in Northern Israel: Who Gets Sick?

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ABSTRACT: **Background:** An outbreak of pertussis occurred in a daycare center with 87.5% vaccination coverage.

Objectives: To assess the effectiveness of the acellular pertussis vaccine and prevention of pertussis after chemoprophylaxis with azithromycin.

Methods: We studied 31 daycare children aged 3–5.5 years exposed to a child with pertussis. Nasopharyngeal swabs were obtained for *Bordetella pertussis* culture and polymerase chain reaction initially, and at days 21 and 60 of follow-up, in cases exhibiting symptoms.

Results: Of the 31 daycare children 6 (19%) tested positive for *B. pertussis* by PCR, 4 of whom had not been vaccinated against the disease. Of the two vaccinated children who contracted pertussis, one had milder symptoms and the other was asymptomatic. The incidence of pertussis was significantly lower in the vaccinated group (2/27) than in the unvaccinated group (4/4) ($P = 0.000$), with efficacy of the vaccine calculated to be 92.5%. Azithromycin chemoprophylaxis was taken only by 14 of the 25 exposed children (56%). On day 21 follow-up, there was no further laboratory-diagnosed *B. pertussis* cases in any of the exposed children, regardless of whether or not chemoprophylaxis was taken.

Conclusions: Based on the children's clinical manifestations and PCR findings a pertussis outbreak had occurred in the daycare center studied. Our findings support the importance of pertussis vaccination since all the unvaccinated children in the daycare center contracted the infection.

IMAJ 2010; 12: 283–286

KEY WORDS: *Bordetella pertussis,* polymerase chain reaction, outbreak

Although the incidence of pertussis has decreased following the introduction of the pertussis vaccine, over the last two decades there has been a resurgence of disease in many countries [1] with some of this resurgence occurring in outbreak settings [2]. Nevertheless, while there are controlled trials of acellular pertussis (DTaP) vaccine efficacy and prospective studies of effectiveness [3], few studies have examined DTaP effectiveness in an outbreak setting, and even fewer have explored the efficacy of macrolide post-exposure prophylaxis in such settings. To address these issues the current study used data collected from individuals exposed to a pertussis outbreak (December 2005 to January 2006) in a daycare center, where a relatively high proportion of children were not immunized.

*** Both authors contributed equally to this study**

PCR = polymerase chain reaction

SUBJECTS AND METHODS

This study was conducted in Israel where, despite vaccination at age 2, 4, 6 and 12 months, there has been an ongoing upward trend in the incidence of pertussis over the last 20 years [4-8]. The study participants were 31 of the 32 attendees and 3 staff members employed at a private, licensed daycare center. The age range was 3.5–5.0 years (mean 4.2 ± 0.8 years) for the children and 26–48 years for the staff members. The daycare center is located in a single building with one large hall functioning as the common play area.

PROCEDURE

The immunization status, reported by the parents as the number of vaccines administered, the demographics and the clinical data were collected via a written questionnaire completed by the parents on day 1. Also on day 1, two nasopharyngeal Dacron swab specimens (Medical Wire, Medeco, Corsham, UK) were collected for culture and PCR from the study participants. All laboratory testing was conducted at Bnai Zion Medical Center's clinical microbiology laboratory. Specimens were immediately plated on charcoal agar plates (Hylabs, Rehovot, Israel) and incubated at 37°C for 14 days. Polymerase chain reaction targeting IS*481* and pertussis toxin primers was performed from the nasopharyngeal specimen using a semi-nested PCR (Proligo LLC, Boulder, CO, USA) [9,10]. The PCR test was considered positive only if both targets tested positive. The laboratory adhered to standard PCR precautions. When pertussis was diagnosed, children were advised to remain at home for 5 days of treatment with azithromycin (10 mg/kg on the first day followed by 5 mg/kg for the subsequent 4 days). Azithromycin was chosen because of its effectiveness and tolerability [11,12]. In addition, on day 1, all contacts of the exposed individuals were

prescribed chemoprophylaxis with the same azithromycin regimen. Follow-up phone calls were conducted on days 21 and 60 to the parents of all 32 children. Moreover, for surveillance of additional cases, parents of initially asymptomatic children were questioned about the development of disease symptoms. Repeat nasopharyngeal specimens for PCR and culture were obtained for children with cough symptoms of at least 4 days duration.

With regard to case definition, Centers of Disease Control criteria were used to categorize cases as confirmed, suspected, or probable [13].

After determining the rate of pertussis in both unvaccinated (0 doses) and vaccinated children, the vaccine effectiveness was calculated. Vaccine effectivness equals [(PRU-PRV) / PRU] x 100 [11].

RESULTS

VACCINE EFFECTIVENESS

Of the 32 daycare children 31 participated in the study. The non-participant was a 4 year old fully immunized child who had no cough symptoms during the study period and whose parents did not wish to be included in the study. Demographic and clinical features of the pertussis cases are summarized in Table 1 and Figure 1. Only 4 of the 31 children in the daycare center were unimmunized despite the fact that these children had no medical contraindication or precaution for pertussis immunization. No further explanations for vaccination refusal were given. The remaining 27 children had all received four doses of acellular pertussis vaccine (Infanrix®, SmithKline Beecham Biologicals). For the daycare children, the time from the last dose of pertussis vaccine to the current outbreak ranged from 2.5 to 4 years. None of the families reported previous illness with pertussis.

Among the 31 children, 6 (19%) fulfilled the case definition for pertussis in an outbreak setting. Four of these six (cases 1–4) were unimmunized and developed a prolonged paroxysmal cough lasting at least 1–2 weeks, thus fulfilling the CDC's confirmed case classification on day 1. The PCR tests for all four of these patients were positive, but their nasopharyngeal specimens were culture-negative. Although suspected on day 1, pertussis was confirmed in one of the vaccinated children solely on the basis of a positive PCR test (case 5). Regarding the other vaccinated child with suspected pertussis (case 6), the case was confirmed on the basis of clinical symptoms and the establishment of an epidemiologic link to another individual with a positive PCR. Overall, pertussis was less prevalent in the fully vaccinated group (2/27) compared with the non-vaccinated group (4/4). Unvaccinated children acquired their pertussis earlier than the vaccinated children [Table 1, Figure 1]. There were no positive pertussis cultures. All staff members denied cough and tested negative for pertussis (culture and PCR). The calculated effectiveness of the acellular pertussis vaccine was 92.5 (95% confidence interval 40–98).

Table 1. Epidemiological, clinical and laboratory findings in pertussis patients

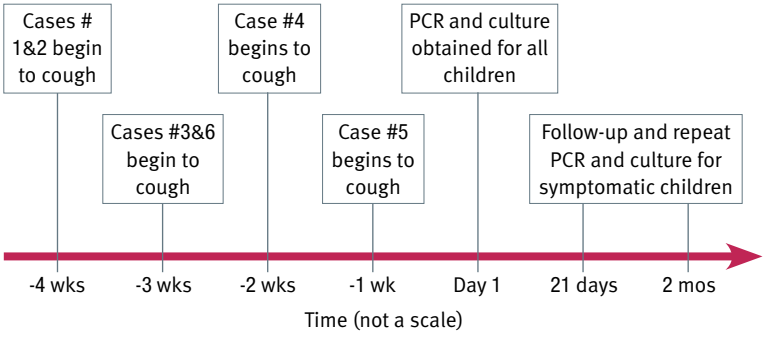
Case no.	Age (yrs)	Duration of cough§	No. of vaccine doses	PCR
1	5.5	4*+	0	Positive
2	5	4*	0	Positive
3	4.5	3*+	0	Positive
4	3	2*	0	Positive
5	3	1*+	4	Positive
6	4.5	3*+	4	Negative

§Duration of cough: 1 = one week, 2 = more than one and less than 2 weeks, 3 = more than 2 and less than 3 weeks, 4 = more than 3 and less than 4 weeks

* Paroxysmal cough

+ Whoop

Figure 1. Timeline of pertussis infection in the daycare center (case# fromTable)



PRU = the rate of pertussis in unvaccinated children
PRV = the rate of pertussis in vaccinated children

EFFICACY OF CHEMOPROPHYLAXIS

On day 1 all children in the daycare center were advised to take azithromycin as chemoprophylaxis according to the regimen noted in the Methods section. For those children with suspected pertussis on day 1, parents were informed that this same prescription was given as treatment rather than prevention. Household contacts of children with confirmed disease were advised to also begin chemoprophylaxis by day 3. Since all the unvaccinated children in the daycare center contracted pertussis and all the exposed children were fully immunized, no booster vaccine was recommended. Phone interviews, conducted with parents on days 21 (first follow-up) and 60 (second follow-up), were used to assess compliance to treatment and chemoprophy-

CDC = Centers for Disease Control

laxis and to identify additional suspected cases. Only 14 of the 25 exposed (vaccinated) children at the daycare center (56%) took the chemoprophylaxis. No adverse side effects were reported during follow-up. Regarding additional case identification, at the first follow-up, 5 of the 25 exposed but undiagnosed children had developed an upper respiratory tract infection with cough (3 of the 5 had completed prophylaxis whereas 2 had declined azithromycin, $P = 0.62$). In all cases, the cough was neither paroxysmal nor accompanied by a whoop. Repeat testing for pertussis among those who developed upper respiratory tract infection was negative. By the second follow-up phone call, all exposed children were asymptomatic.

DISCUSSION

The children's clinical manifestations and PCR findings clearly point to a pertussis outbreak in the daycare center. Our findings reaffirm the importance of pertussis vaccination since all the unvaccinated children in this center contracted the disease. The results reported here indicate that the DTaP vaccine applied in the context of a four-immunization regimen provides effective coverage for recently vaccinated children exposed to a pertussis outbreak. As noted above, the calculated effectiveness of the acellular pertussis vaccine was > 90%. Furthermore, although the limited number of observations in our study precludes any firm conclusions, our data suggest that azithromycin-based chemoprophylaxis may offer a modest added benefit to recently vaccinated children exposed to a pertussis outbreak.

These findings are important because pertussis outbreaks are common and occur in all age groups and in a wide variety of settings, including hospitals, schools, daycare centers, army barracks, nursing homes, etc. [2,15,16]. For example, reports in recent years have documented outbreaks of pertussis in populations of largely unvaccinated children in Afghanistan [17].

Studies in the U.S., Europe and Africa have mainly focused on evaluating the general (as opposed to outbreak-specific) efficacy of different DTaP vaccines after three doses and have found efficacy to be 73–89% [18,19]. Efficacy levels for the whole-cell pertussis vaccine are more variable and tend to be lower than for the acellular vaccine [20], and estimates for both types of vaccines may be even more variable when tested under conditions of household exposure [21] or community-wide outbreak [22,23]. Thus, one possible explanation for the higher effectiveness rate in the current study is that vaccine effectiveness was calculated in the context of a single, community-wide outbreak. Alternatively, the higher rate may be associated with the additional protection afforded by the fourth vaccine dose and because all the children in the current study were only 2–4 years post-vaccination. The latter explanation is consistent with the results of Bisgard's recent study reviewing the high effectiveness of pertussis vaccine among recently vaccinated children [3]. In the epidemiologic investigation of this outbreak, specimens for both culture and PCR were obtained. Notably, the laboratory confirmation of pertussis in this daycare outbreak was PCR-based with no culture-positive specimens. Some comment on the laboratory findings is warranted: *B. pertussis* is a fastidious gram-negative coccobacillus, and its isolation from nasopharyngeal secretions is difficult. Given the relative complexity of proper collection with specific swabs and media, a negative culture does not exclude the diagnosis of pertussis. The yield of *B. pertussis*-positive culture depends in part on when the specimen is taken, since most growth occurs in specimens obtained in the early catarrhal stage of the disease. In this outbreak, for some of the children nasopharyngeal specimens were obtained as late as 4 weeks after the onset of coughing. Finally, it should be noted that the ratio of positive PCR samples to positive cultures ranges from 4 to 6:1, respectively [24].

Regarding the efficacy of azithromycin-based prophylaxis, whereas the current CDC recommendations advise chemoprophylaxis for all household and close contact exposures to pertussis, some countries like Canada and the UK recently narrowed their recommendations. For example, the South Yorkshire Health Protection Unit advises that chemoprophylaxis be administered to vulnerable contacts including neonates, unimmunized or partially immunized infants or children, an individual with a chronic illness, or an immunocompromised host [25]. In their 2005 Cochrane review, Altunaiji et al. [11] conclude that there is insufficient evidence to determine the exact benefit of prophylactic treatment of pertussis contacts. Our findings are consistent with such policy recommendations, namely, among those vaccinated there was no evidence of subsequent disease regardless of prophylaxis status. Specifically, although chemoprophylaxis was recommended for all exposed children, staff and family contacts of exposed children, only 14 of the 25 exposed but non-diagnosed children (all recently vaccinated) took the chemoprophylaxis, with no statistical difference regarding the development of subsequent disease between those children whose parents followed the chemoprophylaxis regimen and those who did not. Nevertheless, given the small number of cases in the population studied, our findings must be interpreted with a high degree of caution. Thus, while these findings may be viewed as further support for the UK's guidelines regarding chemoprophylaxis among fully immunized individuals, pending further research, it remains most prudent to adhere to the CDC-recommended pharmacologic control measures after exposure to pertussis, and particularly, after exposure in the context of an outbreak such as described here.

CONCLUSIONS

The efficacy of the acellular vaccine was 92.5% among young children in a daycare center. While the study was not designed to assess the efficacy of azithromycin chemoprophylaxis in pertussis outbreaks, some possible conclusions can be drawn. The initiation of azithromycin did not afford any added benefit in preventing the development of pertussis among recently vaccinated children exposed to the disease in an outbreak setting, and despite the small number of subjects, this study raises questions about the added value of chemoprophylaxis in this age group.

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Capsule

Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*

Fusarium species are among the most important phytopathogenic and toxigenic fungi. To understand the molecular underpinnings of pathogenicity in the genus *Fusarium*, Ma and co-researchers compared the genomes of three phenotypically diverse species: *Fusarium graminearum*, *Fusarium verticillioides* and *Fusarium oxysporum* f. sp. *lycopersici*. Our analysis revealed lineage-specific (LS) genomic regions in *F. oxysporum* that include four entire chromosomes and account for more than one-quarter of the genome. LS regions are rich in transposons and genes with distinct evolutionary profiles but related to pathogenicity, indicative of horizontal acquisition. Experimentally, the authors demonstrate the transfer of two LS chromosomes between strains of *F. oxysporum*, converting a non-pathogenic strain into a pathogen. Transfer of LS chromosomes between otherwise genetically isolated strains explains the polyphyletic origin of host specificity and the emergence of new pathogenic lineages in *F. oxysporum*. These findings put the evolution of fungal pathogenicity into a new perspective.

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Eitan Israeli

Neurologic Evaluations in Normal-Tension Glaucoma Workups: Are they Worth the Effort?

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ABSTRACT: **Background:** Normal-tension glaucoma is a chronic progressive optic neuropathy of unknown etiology. Neuroimaging workup in these patients is controversial.

Objectives: To determine the value of routine neurologic and neuro-ophthalmologic evaluations in patients with NTG.

Methods: We conducted a retrospective review of all patients diagnosed with NTG in our institution between 2001 and 2006. Neurologic and neuro-ophthalmologic data were evaluated.

Results: Sixty-eight patients were considered suitable for the study (35 males, 33 females; age range 43–90 years). Neurologic and neuro-ophthalmologic findings were normal in all of them. The computed tomography brain scan was normal in 88% and duplex carotid Doppler scan was normal in 92%.

Conclusions: Pathologic findings in neurologic and neuro-ophthalmologic assessments were uncommon in NTG. Therefore, contrary to earlier suggestions, neurologic and neuro-ophthalmologic evaluations in typical normal-tension glaucoma patients appear to have no added value.

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KEY WORDS: intraocular pressure, glaucoma, neurologic evaluation

Normal-tension glaucoma is a chronic progressive optic neuropathy. It is defined as a condition of optic disk cupping and visual field loss resembling that seen in other forms of chronic glaucoma, but the untreated intraocular pressure level is less than 22 mmHg and there is no known cause for these changes [1]. The diagnosis of NTG is usually established by exclusion of other optic neuropathies. The need for neuro-ophthalmologic evaluation in NTG patients is controversial [1-7]. On the one hand, compressive lesions of the anterior visual pathway can cause significant cupping of the optic nerves [8-11], in which case neuroimaging studies are recommended [12]. More recently, it was reported that 6.5% of NTG patients have clinically relevant intracranial compressive lesions involving the anterior visual pathway [11]. On the other hand, the prevalence of abnormalities is low in patients with typical NTG and most of them do not routinely undergo neuroimaging [13,14]. A variety of factors may increase the likelihood of identifying an intracranial mass lesion in atypical cases. These factors include age younger than 50 years, lower levels of visual acuity, neuroretinal rim pallor, and poor correlation between the pattern or extent of visual field loss and optic disk cupping [13].

Because the cause of NTG is not known and in view of the controversy in the literature surrounding the need for neurologic assessment, we correlated changes detected by computed tomography and duplex carotid Doppler in patients diagnosed with NTG in order to determine the benefit of routine neurologic and neuro-ophthalmologic evaluations in the comprehensive workup of NTG patients.

PATIENTS AND METHODS

This retrospective case-control study was approved by the local institutional review board committee. NTG was diagnosed according to the glaucomatous visual fields loss, glaucomatous optic disk cupping and an IOP < 22 mmHg on diurnal curve measurements. CT results were available for 71% of the NTG patients and duplex carotid findings were available for 52%. The prevalence of pathologic findings in the neurologic examinations as well as in the neuro-ophthalmologic evaluations (visual acuity, color vision, pupil reaction), brain CTs and duplex carotid Doppler evaluations were calculated. Correlations between the duplex carotid Doppler and brain CT findings were determined by cross-tabulation, as was the correlation between the findings of neurologic examinations and brain CTs. All patients underwent visual field tests using the white-on-white full threshold or the Fastpac Humphrey 24-2 program.

The patients were divided into three groups according to the severity of disease (i.e., mild, moderate, severe visual field defects) derived from the mean deviation, pattern standard deviation, and Hodapp-Anderson-Parrish severity scores [15]. All patients underwent at least two consecutive visual field tests for evaluating their NTG severity.

NTG = normal-tension glaucoma

IOP = intraocular pressure

STATISTICAL ANALYSIS

The three groups of patients were compared for clinical parameters (pachymetry, maximal baseline IOP, IOP following treatment, and the IOP change following treatment) by a one-way analysis of variance (ANOVA). The Gabriel and the Games-Howell multiple comparison tests were used to determine significant differences between pairs of groups. This analysis was done separately for each eye. The level of significance was set at 0.05, and the SPSS for windows software, version 14.0 (Chicago, IL, USA) was used for the analysis.

RESULTS

Altogether, 33 females (49%) and 35 males (51%) in our clinic were diagnosed between 2001 and 2006 as having NTG. The mean age ± SD of the cohort was 68 ± 11 years (range 43–90). The mean follow-up was 4.6 years (range 2.4–6.6). The mean treated IOP was 12.8 ± -2.6 mmHg (range 7–19 mmHg) and the mean IOP max was 18.3 ± -3.0 mmHg (range 10–21). They all had NTG in both eyes but with different severity in each eye. The prevalence of a neurologic pathology in our case series and the correlation between the findings of the three examined parameters (i.e., neurologic and neuro-ophthalmologic examination, duplex carotid Doppler, and brain CT with and without contrast) were assessed [Tables 1 and 2]. Almost two-thirds of them (41/68, 60%) had undergone neurologic examinations and all had neuro-ophthalmologic evaluations; the results were normal in each case.

Slightly more than one-half of the patients (36/68, 53%) had undergone duplex carotid Doppler: the results were normal or did not exceed 25% arterial narrowing in 33 of them (91.7%). Only three patients had any evidence of pathology on the duplex carotid Doppler evaluation; two (5.6%) had a narrowing of 26–51% and one (2.8%) had a narrowing of 51–75% [Table 2]. Of the 48 patients who had undergone brain CT, 42 had normal results, while some pathology was detected in 6, as follows: 2 (4.2%) had an infarct, 2 (4.2%) had a space-occupying lesion not related to optic pathways, and another 2 (4.2%) had brain atrophy [Table 2]. The two patients with abnormal CTs had lesions which were not situated in a location that might have produced a clinical entity capable of mimicking NTG, thus none of the patients with mass lesions had any characteristics suggestive of atypical NTG.

Table 1. Duplex carotid Doppler examination results

	No. of patients	Percent
From normal to 25%	33	91.7
26–50%	2	5.6
51–75%	1	2.8
Total	36	100.0

Table 2. Computed tomography examination results

	No. of patients	Percent
Normal	42	87.5
Infarct	2	4.2
Space-occupying lesion	2	4.2
Atrophy	2	4.2
Total	48	100.0

DISCUSSION

The aim of this retrospective study was to determine the benefit, if any, of a neurologic evaluation as part of the diagnostic workup for suspected NTG. None of the patients diagnosed with NTG had neuro-radiologic evidence of a mass lesion involving the anterior visual pathway. Our findings clearly showed that the prevalence of abnormalities identified by neurologic examinations, neuro-ophthalmologic evaluations, duplex carotid Doppler examinations and brain CT scans (with and without contrast) was too low to justify their implementation in diagnosing these patients in the absence of other clinical signs and symptoms. Nevertheless, part of the neuro-ophthalmologic evaluation, such as pupil reaction and color vision, might be included in a routine evaluation of optic nerve function by most ophthalmologists and could be performed without further cost. These results indicated that there is no need for neuroimaging in NTG patients who present with typical and uncomplicated NTG. These results and conclusions are quite similar to those of Girkin [16] who advocated neuroimaging only in typical NTG that progressed with severe visual loss and threat to fixation. On the other hand, in contrast to our findings, Ahmed et al. [17] suggested routine imaging in every NTG patient (notably, 6.5% of their patients were found to have intracranial tumors).

We do not think that more sensitive neuro-imaging techniques, such as magnetic resonance imaging, would have any added value. Moreover, the fact that they are also not cost-effective would preclude their use in the ordinary clinical setting. In contrast, we recommend that all patients who present with atypical NTG (i.e., younger than 50 years of age, lower levels of visual acuity, neuroretinal rim pallor, and poor correlation between the pattern or extent of visual field loss and optic disk cupping) undergo these evaluations [13].

The results of our current analyses indicate that routine neurologic workups have no diagnostic value in typical cases of NTG. It is, feasible, however, that an ophthalmologist in clinical practice would take every precaution to avoid placing the patient at risk, and consequently refer the patient for further evaluations, which likely involve exposure to radiation as well as considerable expense. At the same time, there should be awareness that most of these measures will prove to have been unnecessary.

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Capsule

Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5

Metabolic syndrome is a group of obesity-related metabolic abnormalities that increase an individual's risk of developing type 2 diabetes and cardiovascular disease. Vijay-Kumar et al. show that mice genetically deficient in Toll-like receptor 5 (TLR5), a component of the innate immune system that is expressed in the gut mucosa and that helps defend against infection, exhibit hyperphagia and develop hallmark features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance and increased adiposity. These metabolic changes correlated with changes in the composition of the gut microbiota and, importantly, transfer of the gut microbiota from TLR5-deficient mice to wild-type germ-free mice conferred many features of metabolic syndrome to the recipients. Food restriction prevented obesity, but not insulin resistance, in the TLR5-deficient mice. These results support the emerging view that the gut microbiota contributes to metabolic disease and suggest that malfunction of the innate immune system may promote the development of metabolic syndrome.

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Eitan Israeli

Capsule

Cytomegalovirus immune evasion strategy

Cytomegalovirus (CMV) infects a large percentage of the world's population. Although most of those infected are asymptomatic, CMV is a substantial public health concern for immunocompromised individuals and neonates. CMV is unusual in that it can super-infect: it reinfects hosts that are already infected with the virus, even in the presence of a strong, specific immune response. Hansen et al. found that in rhesus macaques, a good model for human CMV super-infection, CMV establishes super-infections by evading the immune response mediated by $CD8^+$ T cells. A series of viral mutants deficient in expression of the US2-11 glycoproteins, which regulate antigen presentation to $CD8^+$ T cells, revealed that, although able to establish the initial infection, these viral mutants were unable to super-infect. Depletion of $CD8^+$ T cells from the monkeys allowed infection by the mutant viruses. These results highlight the difficulties in developing an effective protective vaccine against CMV itself, but suggest that CMV-based vectors may be useful in other vaccine efforts such as those against HIV.

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Eitan Israeli

Prognostic Significance of HER-2/neu Expression in Patients with Ductal Carcinoma In Situ

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ABSTRACT: **Background:** The prognostic significance of biologic markers in women with ductal carcinoma in situ is not fully understood. HER2/neu is a marker of prognostic significance that is routinely assessed in invasive cancer but its correlation with clinical outcome in DCIS is still obscure.

Objectives: To evaluate the significance of HER-2/neu expression as a prognostic marker in DCIS.

Methods: Clinical and pathologic data from 84 patients treated for DCIS were analyzed. HER-2/neu expression was determined by immunohistochemical staining. Histopathologic parameters (nuclear grade, histologic subtype, necrosis, calcifications, margins) were reviewed by an experienced pathologist. Local recurrence and/or metastatic spread were used as endpoints to determine the prognostic significance of HER-2/neu expression.

Results: With a median follow-up of 94.8 months, nine recurrences were reported. Neither univariate nor multivariate analysis showed a significant correlation between HER-2/neu expression and disease recurrence or the time to disease recurrence. Although HER-2/neu expression demonstrated a significant association with high nuclear grade ($P < 0.0001$) and comedo subtype ($P < 0.0001$), there was no correlation between these histologic features and recurrence rate. The correlation between high nuclear grade and disease recurrence approached statistical significance ($P = 0.07$).

Conclusions: No significant association was found between HER-2/neu expression in DCIS and disease recurrence. However, HER-2/neu correlated with negative markers such as nuclear grading and comedo necrosis, and its role should therefore be investigated in larger studies.

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KEY WORDS: HER-2/neu, ductal carcinoma in situ, prognosis, markers, recurrence

In the last decade the incidence of ductal carcinoma in situ has increased, probably related to the introduction of mammography screening. In asymptomatic women, 15–25% of all screening-detected breast cancers are DCIS lesions [1].

DCIS = ductal carcinoma in situ

Treatment guidelines for DCIS have changed over the last two decades and most patients are now treated with breast-conserving therapy. Although these lesions do not metastasize, they carry a relatively high risk for local recurrence and of subsequently developing into invasive carcinoma, particularly when high nuclear grade or comedo-type necrosis is found in the primary lesion [2,3].

Although adjuvant breast irradiation and hormonal therapy reduce the number of ipsilateral breast recurrences by about half [4], local recurrence and the development of invasive cancer are still of concern. In an effort to optimize treatment outcome in these cases, multiple studies have examined the association of various clinical, pathologic, molecular and treatment-related factors with subsequent invasive breast carcinoma or local recurrence. To date, only histologic features like comedo-necrosis, nuclear grade and tumor size have been shown to correlate with local recurrence [4-8].

The last decade has witnessed an increasing number of biologic markers of breast neoplasms. One of the markers with probable prognostic significance is over-expression of the HER-2/neu oncogene within the tumor. Although HER-2/neu over-expression has been shown to correlate with a poor prognosis in invasive breast cancer [9,10], little information is available on the relationship between its over-expression and the outcome of patients with DCIS. Previous studies have indeed demonstrated that HER-2/neu expression correlates with aggressive histologic features in DCIS [11-20], but the prognostic significance of HER-2/neu expression in the clinical setting has yet to be determined.

The purpose of the present study was to assess the correlation of HER-2/neu over-expression and the long-term outcome in DCIS patients, and to evaluate its prognostic significance as well as its association with other histopathologic and biological characteristics.

PATIENTS AND METHODS

The medical records of all patients who underwent breast surgery in our hospital between 1990 and 1998 were reviewed. The study population included patients with the histopathologic finding of DCIS, without invasion. The data

were retrieved using the institution's computerized data bank. All cases diagnosed as DCIS were retrieved and the pathology was revised. Patients were excluded if any type of invasion was found or if the follow-up data were incomplete. Demographic parameters were retrieved from the patients' medical records. Using the outpatient clinic files, we evaluated the clinical course with regard to the development of local recurrence of DCIS or invasive cancer, metastatic spread and mortality. Further data were obtained by means of a telephone interview.

HISTOPATHOLOGIC REVIEW

Histopathologic assessment was performed on paraffin sections stained with hematoxylin-eosin. The following information and pathologic features were recorded for the initial and re-excision specimens:

- Predominant histologic subtype, categorized as comedo, cribriform, papillary, micro-papillary, or solid patterns
- Predominant and highest nuclear grade
- Presence or absence of necrosis
- Presence or absence of micro-calcifications
- Margin status, classified as positive, close, negative or uncertain (when the specimen was not inked or was fragmented such that the specimen margins could not be determined).

Nuclear grade was categorized into two groups: favorable (low or intermediate) and unfavorable (high). Histologic pattern was grouped as comedo and non-comedo subtypes.

CLINICAL OUTCOME

Local recurrence was defined as the reappearance of DCIS or invasive carcinoma in the ipsilateral breast. All recurrences were confirmed histologically. Distal metastases were diagnosed by computed tomography scan, bone scans or ultrasound. When the imaging finding was doubtful, fine needle or core biopsies were performed. All time intervals were calculated from the date of the first diagnosis.

HER-2/NEU EXPRESSION

HER-2/neu oncogene expression was determined by immunohistochemical staining of tissue sections with monoclonal antibodies. The monoclonal antibody used was at a dilution of 1:100 (monoclonal mouse anti-HER-2, Zymed Laboratories, San Francisco, USA). The primary antibody was visualized using biotinylated horse antibody to mouse immunoglobulin and streptavidin-horseradish peroxidase, followed by diminobenzidine solution. Breast carcinomas, positive or negative to HER-2/neu, were used as negative and positive controls, respectively. The specimens were classified as positive or negative solely on the basis of tumor cell membrane reactivity. The percentage of positive cells in the tumor population was determined by visual estimation, and a semi-quantitative analysis of HER-2/neu staining was performed. According to the literature and common practice, the staining pattern was scored as follows:

- 0 = no staining or membrane staining in less than 10% of the tumors cells
- 1+ = faint/barely perceptible membrane staining in more than 10% of the tumor cells with the cells stained only in part of their membrane
- 2+ = weak to moderate staining of the entire membrane in more than 10% of the cells
- 3+ = strong staining of the entire membrane in more than 10% of the cells.

A sample was considered positive for HER-2/neu over-expression if more than 10% of the epithelial cells demonstrated a moderate (2+/3+) to strong (3+) staining of the entire membrane.

STATISTICAL ANALYSIS

The association between over-expression of HER-2/neu and recurrence and all categorical variables (clinical, pathologic, and treatment-related variables) was analyzed using chi-square and Fisher's exact test. Using the *t*-test, mean age was compared between HER-2/neu positive and negative patients and between those with and without recurrence. Kaplan-Meier survival method was used to evaluate survival rates in the various patient subgroups. Log-rank test was performed to compare disease-free time between patient subgroups. Logistic regression and Cox proportional hazards model were applied to study simultaneously the effect of possible risk factors on recurrence and time to recurrence respectively. Statistical significance was accepted at $P < 0.05$. The data were analyzed using SAS software (version 9.1, Cary, North Carolina, USA)

RESULTS

A total of 112 women who underwent surgery between 1990 and 1998 with postoperative finding of DCIS were initially included in the study. Twenty-eight patients were excluded due to inadequacy of the pathologic material, death due to unrelated diseases soon after diagnosis of DCIS, or because of incomplete follow-up. The remaining 84 women were followed for a median period of 94.8 months (range 48–168 months). Mean age of the patients was 55.8 years (range 35–82 years).

In 80 of the 84 remaining patients (95%) the initial treatment was breast-conserving surgery by lumpectomy, and in 4 patients mastectomy was performed as the initial treatment. Twenty-seven patients (34%) from the group treated initially with lumpectomy underwent subsequent mastectomy due to multifocal disease, widespread DCIS within the breast, or due to massively involved margins in the first operation. Another 10 patients (12.5%) from the initial lumpectomy

group underwent further excision of the primary tumor site due to close, positive or uncertain margins. Of the 52 patients finally treated by breast-conserving surgery, 43 also underwent breast irradiation or a combination of breast irradiation and tamoxifen. Of the 31 women who finally underwent mastectomy, 15 received tamoxifen after the operation. All women treated postoperatively with tamoxifen had estrogen receptor-positive tumors.

Table 1. Comparison of recurrence rate by clinical parameters and histopathologic variables

Variable	No. of patients (%)	No. of patients with recurrence (%)	No. of patients without recurrence (%)	*P value*
Nuclear grade				
High	32 (38.1)	6 (18.75)	26 (81.25)	0.07
Intermediate and low	52 (61.9)	3 (5.77)	49 (94.23)	
Histologic pattern				
Comedo type	38 (45.78)	5 (13.16)	33 (86.84)	0.72
Non-comedo	45 (54.2)	4 (8.89)	41 (91.11)	
Margins				
Free	49 (94.23)	7 (14.9)	42 (85.71)	0.4
Not free	3 (5.77)	1 (55.33)	2 (66.67)	
Necrosis				
Present	73 (86.9)	7 (9.56)	66 (90.41)	0.33
Absent	11 (13.1)	2 (18.18)	9 (81.81)	
Calcifications				
Present	66 (78.57)	7 (10.61)	59 (89.29)	1.0
Absent	18 (21.43)	2 (11.11)	16 (88.89)	
Family history of breast cancer				
Present	16 (19.04)	3 (18.75)	13 (81.25)	0.37
Absent	64 (76.19)	6 (9.38)	58 (90.63)	
No data	4 (4.76)			
Menopausal state				
Before menopause	24 (28.57)	2 (8.33)	22 (91.67)	07
After menopause	57 (67.85)	7 (12.28)	50 (87.72)	
No data	3 (3.57)			

Table 2. Comparison of recurrence rate by HER-2/neu expression in surgical subgroups (lumpectomy vs. mastectomy respectively)

HER-2/neu over-expression	No of patients (%)	No. of patients (%) with recurrence	No. of patients (%) without recurrence	*P value*
Lumpectomy with and without adjuvant treatment				
Positive	22 (41.50)	5 (22.73)	17 (77.27)	0.2
Negative	30 (58.50)	3 (10)	27 (90)	
Mastectomy with and without adjuvant treatment				
Positive	15 (48.38)	0 (0)	15 (100)	1
Negative	16 (51.61)	1 (6.25)	15 (93.75)	
Lumpectomy without adjuvant treatment				
Positive	4 (57.14)	1 (25)	3 (75)	0.48
Negative	3 (42.86)	2 (66.6)	1 (33.3)	
Lumpectomy with adjuvant treatment (radiotherapy +/- tamoxifen)				
Positive	17 (37.77)	4 (23.53)	13 (76.47)	0.07
Negative	26 (57.77)	1 (3.85)	25 (96.15)	
Mastectomy without adjuvant treatment	8 (80)	0 (0)	8 (100)	
Positive	2 (20)	0 (0)	0 (0)	
Negative				
Mastectomy and tamoxifen treatment				
Positive	4 (19.06)			
Negative	10 (47.61)	0 (0)	4 (0)	1
Unknown	7 (33.33)	1 (10)	9 (90)	

During the follow-up period, recurrence of disease in the ipsilateral breast was diagnosed in 9 patients (10.7%). The primary surgical treatment was lumpectomy in eight patients and mastectomy in one. Five of these patients had been treated by adjuvant radiotherapy after the local excision and three patients also received tamoxifen. The woman who underwent mastectomy was also treated with tamoxifen.

In two patients the recurrent lesion was DCIS, discovered 22 and 82 months after primary surgery, respectively. These women were subsequently treated by mastectomy and were alive at the end of the study (4 months and 6 years respectively after the recurrence). In another six patients the recurrent tumor was invasive carcinoma, diagnosed between 13 and 142 months after the primary surgery. Four of them underwent subsequent mastectomy and were alive 24–72 months after the second surgery. In the remaining two, the breast tumors were diagnosed simultaneously with distant metastatic disease. One of them was treated with chemotherapy and died shortly after, and the other woman is still alive 8 months after the diagnosis of recurrence.

The woman with recurrent disease who was initially treated by mastectomy for DCIS presented 72 months later with local recurrence of invasive cancer in the mastectomy scar and metastatic spread. She received chemotherapy but died a few months later. Histopathologic revision of the resected breast did not show any focus of invasion.

Two patients died during the study period due to causes other than breast cancer and five patients developed other malignancies.

None of the following parameters – age, menopausal state, family history, resection margins, histologic pattern, and presence of necrosis or micro-calcifications – significantly influenced the rate of disease recurrence. However, a tendency to higher recurrence rates was observed in patients with high nuclear grade tumors ($P = 0.07$) [Table 1].

From among the 84 patients finally included in the study, in 37 (44.05%) the tumors were positive for HER-2/neu over-expression and in 47 patients (55.95%) they were negative. No correlation was found between the recurrence rates in patients positive for HER-2/neu as compared to patients without over-expression of HER-2/neu: 13.5% (5 of 37) versus 8.51% (4 of 47) ($P = 0.46$).

When the patients were grouped according to surgical treatment subgroups (patients who underwent lumpectomy versus patients who underwent initial mastectomy) and subgroups according to adjuvant treatment administration (patients given versus patients not given the treatment), the potential influence of the type of therapy on the outcome was eliminated. No significant correlation was found regarding

the relationship between HER-2/neu over-expression and disease recurrence for these subgroups as presented in Table 2, although patients initially treated by lumpectomy and adjuvant therapy showed an almost statistically significant association between expression of HER-2/neu and disease recurrence ($P = 0.07$).

The association between HER-2/neu and the different histologic features are summarized in Table 3. Most patients (61.9%) had DCIS with favorable nuclear grade. HER-2/neu over-expression was significantly higher in DCIS showing high nuclear grade compared to low nuclear grade (71.8% vs. 26.9, $P < 0.001$). HER-2/neu over-expression was also significantly higher in DCIS showing comedo compared to non-comedo pattern (68.4% vs. 24.4, $P < 0.001$). A tendency to HER-2/neu over-expression was also observed in DCIS lesions showing calcifications ($P = 0.05$).

Familial history of breast cancer did not influence the incidence of HER-2/neu over-expression and no correlation was found between HER-2/neu and the timing of recurrence ($P = 0.58$).

In multivariate analysis using a logistic regression model that included HER-2/neu, clinical variables (menopausal state, age, family history, type of surgery, tamoxifen treatment) and pathologic variables (histologic subtype, nuclear grade, margins), no HER-2/neu or any other marker was found to be of significance, either alone or together with other markers.

Table 3. Correlation between HER-2/neu expression and clinical and histopathologic variables

Variable	No. of patients (%)	No. of HER-2/neu negative patients (%)	No. of HER-2/neu positive patients (%)	*P value*
Nuclear grade				
High	32 (38.10)	9 (28.13)	23 (71.87)	‹ 0.0001
Non-high	52 (61.90)	38 (73.07)	14 (26.92)	
Histologic pattern				
Comedo type	38 (45.78)	12 (31.58)	26 (68.42)	‹ 0.0001
Non-comedo type	46 (54.22)	34 (75.56)	12 (24.44)	
Necrosis				
Present	73 (86.90)	38 (52.05)	35 (47.95)	0.1
Absent	11 (13.1)	9 (81.82)	2 (18.18)	
Calcifications				
Present	66 (78.57)	33 (50)	33 (50)	0.05
Absent	18 (21.43)	14 (77.78)	4 (22.22)	
Family history of breast cancer				
Present	16 (19.04)	10 (62.50)	6 (37.5)	0.5
Absent	64 (76.19)	33 (51.56)	31 (48.44)	
Unknown	4 (4.76)			

DISCUSSION

Since the incidence of non-invasive breast cancer has increased, and as conservative breast surgery became the new standard of care, local recurrence of either DCIS or invasive cancer has become the major concern when treating patients with this potentially curable disease. In the last two decades several studies assessed prognostic markers in a continuous quest for improving outcome. In the NSABP study B-17 moderate-to-marked comedo-necrosis was the single histologic feature found to predict recurrent disease [4]. Solin et al. [5] found that no single histopathologic parameter was associated with local recurrence. Ottesen and collaborators [6] reported that comedo-necrosis, large nuclei, and tumor size > 1 cm were significant predictors of local recurrence after excision alone, and Silverstein et al. and the Van Nuys group [7] created a DCIS pathologic classification based on nuclear grade and the presence or absence of comedo-necrosis. The Van Nuys Prognostic Index builds on this classification with the addition of two parameters with prognostic significance, as identified in multivariate analysis, namely tumor size and margin width [8].

The last decade has witnessed the emergence of increasing numbers of biologic markers to characterize breast neoplasms. These markers typically reflect alterations in genes that regulate cell growth, development and proliferation. The biologic marker profiles of patients with DCIS and their potential prognostic significance are currently being investigated [9,10]. One of the markers with probable prognostic significance is the HER-2/neu oncogene.

The human epidermal growth factor receptor 2 (HER-2)/neu (c-erbB-2) oncogene is localized on chromosome 17q, and encodes a trans-membrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor (EGFR) or HER family [11,12]. Amplification of the HER-2/neu oncogene with the expression of its protein is found in 10–30% of invasive breast carcinomas [13,14] and 30–60% of DCIS cases [14-16]. In invasive breast cancer, HER-2/neu over-expression has been shown to correlate with poor prognosis and poor response to treatment [13,14]. However, the clinical significance of HER-2/neu over-expression in DCIS is presently unknown. In previous studies HER-2/neu expression in DCIS was shown to correlate with aggressive histologic features such as: high nuclear grade, comedo type, the presence of necrosis, and Ki-67 over-expression [10,14-20]. A significant inverse correlation has also been found between HER-2/neu staining and hormone receptor expression in patients diagnosed with DCIS [10,14,15,18]. All these studies showed that HER-2/neu over-expression correlates with various pathologic and molecular factors believed to be associated with a more aggressive behavior, leading to the assumption that DCIS that over-expresses HER-2/neu represents a biologically definable category with prognostic significance.

In the present study we assessed a rather large cohort of DCIS patients with a relatively long follow-up period, to investigate this correlation and to evaluate the prognostic impact of HER-2/neu in DCIS patients. During the study period 10.7% of patients initially operated on for confirmed

DCIS had recurrences. The median follow-up period was 94.8 months, which is longer than in most previous series [6,9,19,21]. The relatively lower recurrence rate in the present study could likely be attributed to the wide use of adjuvant irradiation in our patients.

No significant association between HER-2/neu expression and rate of disease recurrence was found, which concurs with the results of previous studies [14,17,18,22]. In the study reported by Ringberg and co-authors [14], three biological markers were associated with disease recurrence in a univariate analysis: Ki-67 levels, p53 and bcl-2 but not HER-2/neu. Reporting on their 49 patients who were treated with either mastectomy or conservative surgery with or without irradiation, Perin et al. [22] found no significant association between a wide variety of biologic markers including ER, PR, HER-2/neu, and p53, and the rate of disease recurrence. Cornfield and team [17], in a study of 151 patients with DCIS who underwent wide excision and observation alone, also reported no significant association between any of the biological markers evaluated including HER-2/neu and disease recurrence. Roka et al. [18] also found that high nuclear grade and negative estrogen receptor are risk factors for recurrence, whereas other factors like HER-2/neu and p53 did not have any prognostic significance.

Regarding the relationship between HER-2/neu over-expression and histopathologic parameters, the present study demonstrated a significant association between HER-2/neu over-expression and both the high grade and comedo subtypes of DCIS. Similar results were also reported in previous studies [10,15,16,18,19].

When the association between diverse variables and the rate of disease recurrence was analyzed, none of the following parameters – age, menopausal state, family history, resection margin, histologic pattern, presence of necrosis or microcalcifications, presence of microinvasion – significantly influenced the rate of local recurrence. However, as expected, a tendency to higher recurrence rates was observed in high grade DCIS ($P = 0.07$).

Although mastectomy is considered to be curative in the treatment of DCIS, with very low recurrence rates [23], most patients are currently treated by breast-conserving surgery with comparable results in terms of survival. Recurrence rates of 6% to 12% were reported in cases in which adjuvant treatment was administrater after breast-conserving surgery [4,24]. In the present study the outcomes were similar: 3.23% recurrence rate in patients treated by mastectomy, 11.63% in patients treated by breast-conserving surgery and subsequent adjuvant therapy (radiotherapy or/and tamoxifen), and 42.86% in patients treated by local excision alone – further highlighting the benefit of adjuvant therapy in patients treated by local excision.

ER = estrogen receptor
PR = progesterone receptor

In conclusion, although in the present study HER-2/neu was not an independent risk factor for recurrence, it was associated with other risk factors such as comedo subtype and high grade. This paradox was also noted in the previous studies. However, since most studies were relatively small, it is still possible that larger series will reveal the subgroups of DCIS patients in which HER-2/neu will be of value in therapeutic decisions.

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Capsule

Conversion of adult pancreatic α cells to β cells after extreme β cell loss

Pancreatic insulin-producing β cells have a long lifespan, such that in healthy conditions they replicate little during a lifetime. Nevertheless, they show increased self-duplication after increased metabolic demand or after injury (that is, β cell loss). It is not known whether adult mammals can differentiate (regenerate) new β cells after extreme, total β cell loss, as in diabetes. This would indicate differentiation from precursors or another heterologous (non-β cell) source. Thorel and colleagues show β cell regeneration in a transgenic model of diphtheria toxin-induced acute selective near-total β cell ablation. If given insulin, the mice survived and showed β cell mass augmentation with time. Lineage tracing to label the glucagon-producing α cells before β cell ablation tracked large fractions of regenerated β cells as deriving from α cells, revealing a previously disregarded degree of pancreatic cell plasticity. Such inter-endocrine spontaneous adult cell conversion could be harnessed towards methods of producing β cells for diabetes therapies, either in differentiation settings in vitro or in induced regeneration.

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Eitan Israeli

Capsule

Engineering uric acid homeostasis in mice can prevent gout

Not so many years ago, adding a heterologous set of enzymes in order to augment the biosynthetic capacity of a microbe was acknowledged as a remarkable feat of rational design. Apart from the important technical concerns of efficiency and stability, attention then turned to the greater challenge of repairing metabolic dysfunction; the goal here was not only to restore the biochemical reactions but also to place them under endogenous regulation. Kemmer and fellow researchers demonstrate how this might be achieved in mice suffering from excess uric acid, which in humans can lead to the condition commonly known as gout. Uric acid is the product of purine catabolism, and in mice, urate oxidase converts it to allantoin, which is excreted. Excess uric acid can precipitate as the sodium salt, and humans, who lack urate oxidase, cannot tolerate too much of it. Conversely, uric acid can scavenge free radicals, and a moderate amount is deemed to be beneficial. Stitching together a mini-circuit comprising a *Deinococcus* transcriptional repressor and promoter as well as *Aspergillus* urate oxidase enabled these authors to maintain serum uric acid concentration in urate oxidase-deficient mice at normal physiologic levels.

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Eitan Israel

"Everyone is ignorant, only on different subjects"

Will Rogers (1879-1935), Cherokee cowboy, comedian, humorist, social commentator, vaudeville performer and actor

The Israel National Immunization Registry

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ABSTRACT: Immunization coverage is a major health indicator. In Israel, routine childhood immunizations are provided at community public well-baby clinics. Immunization monitoring is an important cornerstone of a national health policy; however, data obtained through sampling carries the risk of under-representation of certain population strata, particularly high risk groups. Despite high national average immunization coverage, specific subpopulations are under-immunized, as highlighted by outbreaks of vaccine-preventable diseases. The mean national immunization coverage at age 2 years (2006 data) was: DTaP-IPV-Hib4 (all 93%), HBV3 (96%), MMR1 (94%), HAV1 (90%). These reports are based on a 17% population-based sample in some districts and on cumulative reports in others. A national immunization registry requires data completeness, protection of confidentiality, compulsory reporting by providers, and links to other computerized health records. It should provide individual immunization data from infancy to adulthood and be accessible to both providers and consumers. In 2008 the Israel Ministry of Health launched a national immunization registry based on immunization reporting from well-baby clinics using a web-based computerized system. As of January 2010, 120 well-baby clinics are connected to the nascent registry, which includes the records of some 50,000 children. The implementation of a comprehensive national immunization registry augurs well for the prospect of evidence-based assessment of the health status of children in Israel.

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KEY WORDS: immunization coverage, children, national register, vaccine-preventable diseases, public health

The prevention and control of vaccine-preventable communicable infectious diseases is a cornerstone of primary prevention in public health. Immunization coverage is considered a major health indicator because it is a sensitive measure of the susceptibility to vaccine-preventable diseases and also indicates the level of protection in a defined population [1-6]. Childhood immunization coverage is defined by the World Health Organization and the United Nations Children's Fund as a comparable indicator reflecting the state of health of children worldwide [7,8]. High quality data are essential to improve immunization coverage and accurately monitor progress towards Millennium Development Goal 4 – namely, a reduction in child mortality [9].

Passwell and Spirer [10] stated that successful immunization programs are the most efficient, cost-effective means to prevent infectious diseases and are therefore a major component of quality health care in Israel and worldwide. Monitoring immunization coverage is an important part of public health surveillance. Moreover, evaluation of immunization coverage enables international comparisons with the goals set by the WHO towards the control, elimination and eradication of communicable diseases [11,12].

GOALS AND DEFINITIONS OF IMMUNIZATION COVERAGE MONITORING

Immunization coverage is defined as the proportion of immunized children in a defined area of the total candidate population of children who constitute the target population. The population definition may change according to the objectives and purposes of the evaluation [13-15]. The target population may be retrieved from databases such as: a) the official demographic registry of live births, b) the population of infants aged 12 months, c) the population of children registered in the national health insurance files or databases of health insurers and/or providers, d) the population of children registered as residents in a defined area, or e) the population of children who are registered or who applied for registration in educational facilities.

The risk approach to estimating immunization coverage is aimed at detecting low coverage in specific geographic areas and population subgroups, thereby enabling further investigation of possible causative factors for under-immunization. Such causes may be related to processes and determinants of the health ser-

DTaP = diphtheria-tetanus-acellular pertussis
IPV = inactivated polio vaccine
Hib = *Haemophilus influenzae* b
HBV = hepatitis B virus
MMR = measles-mumps-rubella
HAV = hepatitis B virus

WHO = World Health Organization

vices structure (accessibility, convenience) or to the special characteristics of the subgroups, including beliefs and perceptions affecting compliance with immunizations [16]. Identification of under-immunized populations and the causative factors enables the planning of action to improve utilization of health services based on principles of health promotion, improved outreach activities within the communities, use of various educational and information tools, and allocation of personnel and designated financial resources [17,18]. In a recent Israel Medical Association national survey of parental attitudes and compliance with routine childhood vaccinations, 90% of parents stated that they vaccinate their children and 87% trust doctors' vaccination recommendations [19]. The missing 10% should be the area of concern.

Immunization coverage is a major health indicator reflecting the level of protection against vaccine-preventable diseases in a defined population

At an individual level, assessment of immunization coverage can provide updated information on a person's immunization scheduling, vaccine indications and contraindications, and side effects and adverse events. These data should be incorporated in the personal medical file. Individual immunization monitoring also facilitates appointment scheduling, and provides information for health care professionals and parents.

Immunization coverage data are drawn from routine administrative reporting by countries, through health management information systems and specific surveys, to produce estimates of national coverage of selected vaccines. These data are processed and used by the WHO, UNICEF, GAVI (the Global Alliance for Vaccines and Immunization), governments, and other immunization stakeholders. There is, however, evidence from various reports and studies demonstrating the lack of accuracy in immunization coverage estimates [20,21]. Furthermore, global, regional and national figures mask inequities of access to immunization within specific groups, for example, socioeconomically deprived or minority groups.

Despite high average national immunization coverage, specific sub-populations are under-immunized, as highlighted by recent outbreaks of vaccine-preventable diseases

METHODS OF MEASURING IMMUNIZATION COVERAGE WORLDWIDE

The methods of measuring immunization coverage differ, based on the sources of information and the surveillance methodologies [4,17,22]. Surveys based on sampling of households have been developed by the WHO. The most useful is the Demographic and Health Survey which is used in developing countries and in some developed countries [23].

UNICEF = United Nations Children Fund

Most developed countries use cumulative administrative data based on health care providers' reports. Full-reporting is usually based on a national computerized database that includes all children in a certain country (registry based). Alternatively, sample-based reports are used by governmental agencies through periodic, random and statistically representative samples. Many western countries perform periodic sample-based immunization coverage surveys. Registries exist in Australia, the United Kingdom and several Canadian provinces [13,24,25]. In the United States, a combination of regional immunization information systems and a national sample-based immunization survey is used [26-28]. The Netherlands, Norway, Portugal and Sweden also maintain national registries; Spain combines regional and national sample-based data [29]. France utilizes a provider-based reporting system [30] [Table 1].

Statistical analysis of the data includes descriptive epidemiologic analysis of immunization coverage and trends with respect to time, place, population groups, schedules, and types of vaccines. It involves various comparisons using epidemiologic analysis, looking for immunization under-coverage, and variables and factors that might be associated with disparities.

IMMUNIZATION COVERAGE MONITORING IN ISRAEL

In Israel, the universal immunization program for the routine administration of childhood immunization is provided in public well-baby clinics across the country. The program includes vaccines against diphtheria, pertussis, tetanus, poliomyelitis, *Haemophilus influenzae* type b, measles, mumps, rubella, varicella, hepatitis B and hepatitis A. Booster doses are provided via the school health services. Pneumococcal conjugate vaccine was introduced in July 2009. The introduction of the rotavirus vaccine is planned for 2010 and of human papilloma virus vaccine for 2011. These vaccines are currently available through health care providers. Influenza vaccination in childhood is recommended and is also available through health care providers. The immunization program in Israel, based on recommendations from the Ministry of Health advisory committee on immunization, is presented in Table 2 [33].

Immunization coverage information is provided by the well-baby clinics to the subdistrict and district health offices in the country. A national report is subsequently issued and the coverage data are reported to the international agencies.

During the years 2000–2008 there was an annual average of 145,000 live births in Israel. The average national immu-

Table 1. Methods of monitoring immunization coverage in 11 developed countries

Country	IC registry	IC survey	IC monitoring system	Population	Ref.
Australia	National, computerized	–	Australian Childhood Immunization Register (ACIR)	Covered by Medicare, 0–17 yrs	[13]
Canada	Provincial/territorial, computerized	National Immunization Coverage Survey	National Immunization Strategy (NIS)	Covered by mandatory health insurance, 0–18 yrs	[25]
France	National, manual	National	Carnet de santé/Carnet des vaccinations Health Certificates. Institute de Veille Sanitaire (InVS)	Reported by doctor as vaccinated, 24 mos	[29,30]
Germany	–	National, annual	German Health Interview examination Survey (KiGGS)	Representative sample, 2–17 yrs	[29]
Netherlands	National, computerized	–	National Immunization Program (NIP)	Population-based registry, all ages	[29]
Norway	National, computerized	–	Norwegian National Immunization Registry (SYSVAC)	Population-based registry, recorded from first vaccination, all ages	[29,31]
Portugal	Local/nationally linked, computerized	–	National Health Information system	Population-based, attending local public health centers	[29]
Spain	Local	National	Administrative	0–18 yrs	[29]
Sweden	National, computerized	Annual National Survey, 24–35 mos, at school	National Surveillance Register, SmiNet	Children registered at Child Health Care Centers	
Taiwan	National, computerized	–	National Immunization Information System (NIIS)	Covered by National Health Insurance	[32]
United Kingdom	National, computerized	National	Cover of Vaccination Evaluated Rapidly (COVER)	Registered with GP, 0–18 yrs	[24,29]
United States	State/City, computerized	National Immunization Survey	Immunization Information System (IIS)	0–6 yrs	[27,28]

IC = immunization coverage

Table 2. Routine immunization schedule for infants and children, Israel, 2009

	First year					Second year		Third year	School		
	Birth	1 mo	2 mos	4 mos	6 mos	12 mos	18 mos	24-30 mos	6 yrs (1st grade)	7 yrs (2nd grade)	13 yrs (8th grade)
Hepatitis B	HBV	HBV			HBV						
Inactivated poliovirus			IPV	IPV	IPV	IPV				IPV	
Diphtheria-tetanus-acellular pertussis			DTaP	DTaP	DTaP	DTaP				dTap	*dTap
Haemophilus influenzae b			Hib	Hib	Hib	Hib					
Pneumococcal conjugate			PCV7	PCV7		PCV7					
Measles-mumps-rubella						MMR			MMR		
Varicella						Var			Var		
Hepatitis A							HAV1	HAV2			

*Children who have not received a booster dose of DTap since the age of 7 years

HBV = hepatitis B vaccine, IPV = inactivated polio vaccine, DTaP = diphtheria-tetanus-acellular pertussis vaccine for use in children, dTap = diphtheria-tetanus-acellular pertussis vaccine for use as booster in children and adults, Hib = *Haemophilus influenzae* b vaccine, PCV7 = Pneumococcal conjugate vaccine, MMR = measles-mumps-rubella vaccine, Var = varicella vaccine, HAV = hepatitis A vaccine

nization coverage at age 2 years in 2006 (the most recent available data) was: DTaP-IPV-Hib4 (all 93%), HBV3 (96%), MMR1 (94%), and HAV1 (90%) [34]. Reporting is dependent on a 17% population-based sample in some districts and on cumulative reports in others. Notably, children who are not registered in the well-baby clinics are not included in the reports. Despite a high national average coverage, specific subpopulations are under-immunized and under-reported, as highlighted by recent outbreaks of vaccine-preventable diseases such as measles [35]. Sampling carries the risk of under-representation of some population strata, particularly high risk groups. Comparison of coverage rates among districts and aggregation of districts' estimates for national reporting are hindered by differences in coverage assessment methods and the absence of regular and consistent data collection.

A national registry of immunization coverage in Israel was designed to:

- Compare the demographic database with the immunization coverage database and identify populations across Israel with low immunization coverage and inadequate utilization of preventive well-baby services. This will facilitate investigation of causative factors and planning of appropriate public health intervention programs.
- Provide information on age cohorts (annually cross-sectional at age 1, 2, 3 years and in the first, second and eighth school grades) and populations, vaccine-specific coverage, and up-to-date or on-time immunization.
- Monitor national immunization coverage trends over time, thus contributing to the evaluation of programs and progress towards the achievement of targets.
- Facilitate sharing of immunization information among districts, while enhancing the reporting capabilities of immunization registries or other information systems.
- Lay the groundwork for the eventual development of a comprehensive immunization registry network covering the entire population – pediatric and adult, which is a key objective of the National Immunization Strategy.
- Be part of the national computerized health records initiative.
- Provide individual-based immunization data from infancy to adulthood to providers, health care professionals and consumers.
- The national immunization registry requires data completeness, protection of confidentiality, compulsory reporting by health service providers, and links to other computerized records.

THE ISRAEL NATIONAL IMMUNIZATION REGISTRY

In September 2008 the trial of a computerized national childhood immunization registry (known by the Hebrew acronym *"Raheli" – Rishum Hisunim Leumi Yisrael*) was implemented as a pilot project in seven Ministry of Health well-baby clinics (one in each district). The system is based on registering each child individually during the initial clinic visit, using entries extracted from the national population demographic database of the Ministry of the Interior. The link to the Ministry of the Interior database ensures unique automatic identification of the child, thereby preventing database entry errors. In the event that a child is not registered in the Ministry of the Interior database (e.g., children of foreign workers and tourists), passport numbers or serial numbers can be used. Every child residing in Israel is entitled to receive health services in the public well-baby clinics, regardless of the legal civil status of the family. As of 1 January 2010, all well-baby services are provided free of charge. Previously, a copayment fee was employed, although impecunious families were exempted from paying.

In 2008 the Ministry of Health in Israel launched a national computerized web-based immunization registry based on reporting from well-baby clinics

The immunization program in the computerized system includes details of the vaccines used in Israel (generic and trade names, batch numbers) held in an enterprise resource planning-based system (System Analysis and Program Development, Walldorf, Germany), and those data are automatically imported into the register. This again avoids errors in data entry. The program is updated in accordance with the Ministry of Health immunization schedule. Extremely stringent precautions and safeguards to ensure data protection are *sine quibus non* in the system.

As of January 2010, over 120 Ministry of Health well-baby clinics report online. To date, the system holds records of some 50,000 children. The aim is to eventually connect all health services in Israel that provide immunizations, thereby enabling health providers and consumers to access individualized, up-to-date information on the immunization status of every person at any age and in any medical facility.

In the future, the Ministry of Health will be able to obtain invaluable data and reports concerning various immunizations on a variety of population cross-sections (age, socioeconomic groups, geographic area, etc.), which will expedite planning and interventions as needed.

Policy makers are becoming more aware of the importance of preventive health programs in general and immunizations in particular. In July 2009, during the term of the 18th Knesset (Israeli parliament), a significant amendment was introduced to Para 66 of The Public Health Ordinance (1940), specifying that the Minister of Health is to establish a national immunization registry [36]. Individual data on immunization status may be used to create an incentive program involving Child Endowment Allowances [37].

The implementation of the national immunization registry has been greeted with enthusiasm by the staff of the well-baby clinics. It has been found to be user-friendly and informative. This important step towards improving vaccination coverage augurs well for the future of public health data collection and analysis in Israel.

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"When you get to the end of your rope, tie a knot and hang on"

Franklin D. Roosevelt (1882-1945), 32nd President of the United States and a central figure in world events during the mid-20th century, leading the U.S. during a time of global economic crisis and world war. Known by his initials, FDR took office in the depths of the Great Depression. His combination of optimism and economic activism is often credited with keeping the country's economic crisis from developing into a political crisis. He named his approach to the economic situation the New Deal.

"In the end, we will remember not the words of our enemies, but the silence of our friends"

Martin Luther King Jr. (1929-1968), American clergyman, activist and prominent leader in the African American civil rights movement. It was during the 1963 March on Washington that King delivered his "I Have a Dream" speech. There, he raised public consciousness of the civil rights movement and established himself as one of the greatest orators in U.S. history. In 1964, King became the youngest laureate of the Nobel Peace Prize, which he received for his work to end racial segregation and discrimination through civil disobedience and other non-violent means. He has become a human rights icon.

Red Reflex Examination in Neonates: The Need for Early screening

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KEY WORDS: Vision 2020, congenital ocular anomalies, congenital cataract, red reflex

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Children are a priority in "Vision 2020," the World Health Organization's global initiative for the prevention of avoidable visual impairment [1]. Congenital cataract is the leading cause of preventable childhood partial sight or blindness [2] – both by primary prevention, for example, through a rubella immunization program, and by secondary prevention depending on early screening of the pupillary red reflex. Given that the optimal time to remove a dense congenital cataract in an infant and initiate optical treatment appears to be age 4 to 6 weeks [3], screening to ensure prompt early treatment is essential for improving visual outcome [4]. Newborn screening for media opacities, comprising examination of red reflex, is widely accepted. The red reflex test uses transmission of light from an ophthalmoscope through all the normally transparent parts of a subject's eye. Any factor that impedes or blocks this optical pathway will result in an abnormality of the red reflex. Corneal opacities, aqueous opacities, iris abnormalities, cataracts, vitreous opacities, and retinal abnormalities including tumors or chorioretinal colobomata, may produce abnormalities or asymmetry of the red reflex [5].

Screening newborns with the red reflex test is widely accepted. The American Academy of Pediatrics [6] and the British Paediatric Association [7] currently recommend red reflex assessment as a component of the eye evaluation in the neonatal period and during all subsequent routine health supervision visits. The purpose of this policy statement is to minimize the risk of delay in the diagnosis of serious vision-threatening or life-threatening disorders. Despite these worldwide recommendations, there are large variations in the implementation of red reflex examination [8-12]. In the UK, less than half the cases in the 1995–1996 cohort of congenital and infantile cataract were detected by screening examinations at age 8 weeks or less [8]. Similarly, a recent study of infantile cataracts in the United States showed that 38% were diagnosed after age 6 weeks [9]. Reporting their 10 year experience in a single regional ophthalmology center, Sotomi et al. [10] showed that none of the 27 infants with congenital cataracts was diagnosed by the newborn screening examination. Six of 8 infants who were diagnosed before 3 months of age had a good visual outcome in contrast to only 3 of 19 diagnosed after 3 months. Considering that this practice was not evaluated prospectively, it is not clear whether the low detection rate is the result of non-compliance, inadequate technique, or low sensitivity of the red reflex test as a screening tool.

In this issue of *IMAJ*, Eventov-Friedman and colleagues [14] report a single-center clinical experience following implementation of the red reflex test as part of the newborn physical examination. During the 2 year study period, of 11,500 newborns who were screened with red reflex examination, 12 were referred to ophthalmology consultation due to suspected abnormal red reflex. In 5, the diagnosis of congenital cataract was confirmed, giving an incidence of 4.3 per 10,000 newborns. Based on routine notification systems for monitoring congenital anomalies in the USA and Europe, the current annual birth prevalence of congenital or infantile cataract has been estimated to be approximately 1 per 10,000 of the total number of births. The British Congenital Cataract Interest Group reported a cumulative incidence of congenital and infantile cataract of 2.29 per 10,000 by age 1 year [13]. In comparison to these findings, the reported incidence in the current study is higher, suggesting a high detection rate. However, in the absence of follow-up or national surveillance for the diseases, the incidence and sensitivity of red reflex have yet to be determined. The positive predictive value (42%) that was shown here, after a short period of implementation, is also higher than that shown elsewhere [15], yet over-referral is unavoidable in the effort to detect infants with congenital media opacities.

The authors included in their report a survey of all Israeli neonatal departments on the implementation of the red reflex test in the newborn examination, showing that until December 2008 only 12 of 26 neonatal departments routinely assessed the red reflex prior to discharge. The disparity between the incidence of congenital cataract in the current study and the 0.68 cases per 10,000 newborns reported to the Israeli registry of congenital anomalies during the years 2000–2008 (personal communication) should encourage neonatologists to implement this screening

for early diagnosis of eye pathology. The Israel Neonatology Association guidelines for routine red reflex examination in newborns published in July 2009 [16] constitute the first step for improving the quality of red reflex screening. However, more specific guidance regarding the purpose and content of red reflex examination and the promotion of programs for training all involved in its management are required. In addition, there is a need for repeated examination before age 6 weeks. In all infants with a family history of retinoblastoma or cataract, neurologic or metabolic disorders, and microphthalmia or eyelid hemangioma, consultation with an experienced ophthalmologist should be emphasized.

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Capsule

Activin A or Tie2 signaling ameliorates osteolytic bone disease

Bone metastases are a common feature of many advanced-stage cancers and are among the most painful and debilitating complications. Tumor cells alter bone tissue by unbalancing the bone remodeling process that occurs naturally throughout adult life. In the context of osteolytic (bone-destroying) metastases, this disruption occurs through an enhanced production of osteoclasts (the cells that resorb bone) – or through a suppressed production of osteoblasts (the cells that build bone). The molecular mechanism by which tumor cells alter the abundance of these cell types is the subject of two recent studies using mouse models of cancer. Vallet et al. (*Proc Natl Acad Sci USA* 2010; 107: 5124) found that multiple myeloma cells cause bone marrow stromal cells to secrete activin A, which is a member of the transforming growth factor-β family of cytokines and which inhibits the differentiation of cells into osteoblasts. In independent work on breast cancer-associated bone disease, Min et al. (*Cancer Res* 2010; 70: 2819) found that Tie2, a receptor tyrosine kinase that is expressed at high levels in breast cancer, is also expressed in bone marrow cells that normally differentiate into osteoclasts and is in fact required for osteoclast production. Inhibition of either activin A or Tie2 signaling with soluble decoy receptors led to the amelioration of osteolytic bone disease, suggesting that these two molecules may be useful therapeutic targets.

Eitan Israeli

"Men occasionally stumble over the truth, but most of them pick themselves up and hurry off as if nothing ever happened"

Sir Winston Churchill (1874-1965), British politician known chiefly for his leadership of the United Kingdom during World War II. He served as Prime Minister from 1940 to 1945 and again from 1951 to 1955. A noted statesman and orator, Churchill was also an officer in the British Army, a historian, writer and artist, and laureate of the Nobel Prize in Literature,

Ritual Circumcision and Urinary Tract Infection in Israel

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KEY WORDS: urinary tract infection, circumcision, febrile infants, hemostasis

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Brit milah, the ritual circumcision of male infants in Judaism, is an obligatory commandment performed on the eighth day of life. This procedure is also practiced in other religions and cultures. Most circumcised males in the world are Muslim. Global estimates suggest that one in every three males worldwide is circumcised [1]. According to advocates of the procedure, circumcision may also provide health benefits to males, the most prominent being prevention of human immunodeficiency virus. It was recently shown in randomized controlled trials that adult circumcision reduced the risk of acquiring HIV infection [2-4]. Many studies have shown that male circumcision also protects against urinary tract infections in infants and children [5-9].

A large survey demonstrated that the incidence of UTI in circumcised male infants was significantly lower than in those who were uncircumcised, 0.02% vs. 0.24, respectively [10]. However, Israeli data of the last 25 years have shown an unexpected high peak in the incidence of UTI in circumcised infants during the first 3 weeks after circumcision [11-15]. The estimated incidence in circumcised male infants was 0.67% [15], higher than in uncircumcised infants in the United States [10].

Why do these observations derive only from Israel data? We found that the main difference between the American reports on the low incidence of infantile UTI after circumcision and the high incidence in Israel is associated with the person who performs the circumcision. In the U.S., most procedures are performed by physicians, while in Israel a *mohel*, an individual with no medical education, is trained to perform circumcisions. In two studies, the calculated odds ratio of acquiring UTI in children circumcised by a mohel compared with those circumcised by a physician were 4.3 and 2.8 [14,15].

In this issue of *IMAJ*, Toker and colleagues [16] report their experience with febrile circumcised infants admitted to the pediatric emergency room of a large Jerusalem hospital. The foremost results of their study were the high rate of UTI in febrile circumcised male infants after day 8 of life, 24.7% compared to 8.4% in females, and that most UTI episodes occurred within 9 days after circumcision. These findings strengthen the observation that ritual circumcision is an important factor in neonatal UTI.

The most striking finding in this study was the high rate of bacteremia in the infected infants, 16.6% with the same microorganism as in the urine, with two of them developing bacterial meningitis. Is this significant infection preventable? By examining the data comparing circumcisions performed by a physician or a mohel in Israel, it appears that the UTI rate is lower when the procedure is performed by a physician. The reasons for the high rate of UTI after a traditional circumcision performed by a mohel are unclear, but it is assumed that the technique of achieving hemostasis may be the main factor leading to urinary tract infections. The mohel uses a gauze dressing wrapped around the penile shaft, whereas physicians apply slight local pressure and calcium-sodium alginate fibers that melt within a few hours. The wrapped gauze used by the mohel has the potential to become resistant to the urine flow, which subsequently leads to urine retention. Urine retention is a known predisposing factor for UTI. One study reported that infants with UTI, after a traditional circumcision performed by a mohel, wore the gauze dressing for 25.6 hours compared to infants without UTI (16.6 hours) ($P = 0.007$) [14].

Consequently, we suggest that ritual circumcisers adopt the hemostasis technique used by physicians, or at least shorten the duration of the shaft wrapping. In conclusion, the unique phenomenon of the high rate of UTI in male infants in Israel seems to be related to the traditional technique of hemostasis. It is time to improve the practice of traditional circumcision.

HIV = human immunodeficiency virus
UTI = urinary tract infection

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Capsule

A pathway to leukemia

Leukemia is initiated and maintained by a small number of self-renewing cells called leukemia stem cells (LSCs), which share properties with hematopoietic stem cells (HSCs), the self-renewing cells that produce healthy blood cells. Wang et al. studied mouse models of acute myelogenous leukemia (AML), a disease that is often refractory to existing therapies. Activation of the Wnt/β-catenin signaling pathway was required for efficient oncogene-mediated conversion of HSCs into LSCs. This pathway is among the most well-studied signaling pathways in cell biology, setting the stage for testing of β-catenin signaling antagonists in preclinical models of AML.

Science 2010; 327: 1650

Eitan Israeli

Capsule

1918 influenza virus matches 2009 H1N1 strain

The "novel" H1N1 swine influenza virus that last year caused the first human pandemic in four decades has one feature that is hardly novel: Its surface protein, hemagglutinin (HA) – which spikes cells and starts an infection – closely matches the HA in the H1N1 virus responsible for the 1918 pandemic. Separated by 91 years, the two strains of the highly mutable virus should be vastly different. This newfound similarity answers many mysteries about the 2009 pandemic, including why it largely spared the elderly.

The new findings, reported online in *Science* (2010; 327: 1563) and *Science Translational Medicine*, also suggest intriguing explanations for how the 1918 influenza virus has evolved since it swept across the globe in several waves, killing more than 50 million people by the winter of 1919. And the investigators are proposing provocative – some say far-fetched – vaccination strategies to preempt future pandemic.

Eitan Israeli

Capsule

Moving signals in breast cancer metastasis

Many types of human breast cancers overexpress a cell-surface receptor – EphA2 – a tyrosine kinase activated by the ligand ephrin-A1 present on adjoining cells. Salaita and co-authors studied the regulation of mechanically stimulated EphA2 signaling by inducing intermembrane signaling between living EphA2-expressing human breast cancer cells and supported membranes displaying laterally mobile ephrin-A1. When the receptors engaged their ligands, they formed clusters that moved radially to the junction between the cells and the membranes. Physically impeding this movement altered the cellular response to ephrin-A1. Different breast cancer cell lines showed differences in receptor movement that correlated with their invasion potential and might indicate their capacity for metastasis formation.

Science 2010; 327: 1380

Eitan Israeli

An Additional Piece in the Israeli Celiac Disease Puzzle

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KEY WORDS: celiac disease, adult, Israel, prevalence

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In the present issue of *IMAJ*, Israeli et al. [1] explore the prevalence of celiac disease in 850 healthy military recruits screened in 2003 by serology and confirmed by intestinal biopsies. The overt CD prevalence, prior to recruitment, was 0.12%. The serologic prevalence was 1.1% and the pathology-based prevalence 0.7%. The authors should be congratulated for undertaking such a task since crucial information on the incidence and prevalence of this frequent autoimmune disease in Israel is lacking. Nevertheless, several controversial aspects arise from the study and will be discussed below.

The prevalence of a disease is the proportion of the affected subjects at a specific time. Prevalence studies should be based on a random sample of the population in the electoral roll or selected by postcodes using the method of population in proportion to size. Unfortunately, most prevalence studies on CD were conducted on sera from selected groups such as blood donors, school-age children, outpatient clinics, or military personnel or recruits as in the present study. This selectivity induces bias to the true prevalence of CD. Table 1 summarizes the studies of CD prevalence done in Israel on selected populations. The table illuminates the bias of comparing the prevalence in selected populations as shown by the wide variation in the prevalence of CD.

In addition to selecting the population to be screened is the dilemma – what serologic test to use. In the last few years the armamentarium of tests expanded considerably. The old anti-gliadin antibody [5] was replaced by the more recent anti-endomysial and anti-tissue transglutaminase [6,7]. The latest to enter the race for the serologic diagnosis or future screening of CD are the new anti-deaminated gliadin peptide and the tTG-neo-epitope. The most commonly used antibody for population screening is immunoglobulinA-EmA and IgA-tTG and only the future will show if newer antibodies like deaminated gliadin peptide or the neo-epitope of tTG will perform better. A major step forward is the replacement of the cumbersome, operator-dependent immunofluorescent EmA by the simpler, standardized, less costly and reliable EmA measured by the enzyme-linked immunosorbent assay technique [6].

Reliance on serum IgA-EmA as the only screening antibody has led to the underestimation of the true prevalence of CD, and adding IgA-tTG improves the results [8,9]. On the other hand, confirmation with EmA is advised when tTG is performed as a first-level screening for suspected celiac disease [10]. In our screening of blood donors, we concluded that the disparity between the various serologic markers suggests that using one serologic marker is insufficient for establishing the true prevalence of CD [4].

The emergence of CD-specific antibodies of the IgG type – namely IgG-EmA, IgG-tTG, IgG anti-deaminated gliadin-analogous fusion peptides [11], celiac G+ antibodies [12], and IgG tTG-neo-epitope [13] – as separate kits or as part of a multiplex immunoassay [14] brings hope to the problem of diagnosing CD in IgA-deficient patients. In fact, IgA deficiency occurs in 1:400 in the general population but 1:40 of celiac patients, increasing the diagnostic importance of this subpopulation. In a recent cost-effectiveness analysis, Dorn et al. [15] concluded that routinely screening for IgA deficiency in order to avoid a false-negative diagnosis is quite costly. It is possible that the screening method of the future will include a multiplex kit with two IgA and two IgG-based, reliable, celiac-specific antibodies, thus avoiding routine total IgA determination.

The issue of mass screening for CD is highly controversial and the reader is referred to the summary of the pro and cons in the September 2009 issue of the

CD = celiac disease

EmA = anti-endomysial antibodies
Ig = immunoglobulin
tTG = anti-tissue transglutaminase

Table. Studies of CD prevalence in Israel on selected populations

Reference	Pathologic confirmation	Overall prevalence (%)	Serologic prevalence (%)	Population	No. screened
1	6\6	0.7	1.1	Military recruits	850
2			1.486	Military personnel	538
3	1\1	0.48	0.48	Normal controls	210
4	10\30	0.6	3.8	Blood donors	1571

British Medical Journal [16,17]. However, we recently learned that 90% of CD is being missed [18], positive serologic results are often not confirmed histologically [19], undiagnosed CD is associated with a nearly fourfold increase in mortality [20], and many of the patients identified by screening do not ultimately benefit from being screened [21]. Clearly, we need a large-scale screening to detect the true prevalence of CD in the population and a centralized national registration system to establish a data bank of CD. The most reliable detecting antibodies and the preferred technique should be established before large-scale screening. Due to the low compliance – for multiple reasons – with a gluten-free diet, new therapeutic strategies to treat CD should be developed [22].

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Capsule

SCMH1 protein provides insurance against oxygen deprivation and tolerance to subsequent ischemia

Individuals suffering a stroke show various degrees of neurological dysfunction due to brain tissue that has been deprived of oxygen. Strategies to minimize damage from this common problem are needed, prompting investigators to focus on a process whereby the resistance of neurons to ischemia (or loss of blood flow) is increased if cells were exposed previously to a mild bout of ischemia insufficient to cause permanent damage. Stapels and colleagues conducted a systematic search for proteins that showed increased abundance in such resistant neurons and identified SCMH1, a mouse homolog of *a Drosophila* polycomb group protein. SCMH1 can modify histones and is thought to function by repressing transcription. In a mouse neuroblastoma cell line, depletion of SCMH1 diminished the induction of tolerance to ischemia, and over-expression of SCMH1 promoted tolerance even in the absence of a conditioning ischemic event. SCMH1 associates with the promoters of two potassium ion-channel genes, and inhibiting the transcription of these genes was sufficient to produce tolerance to subsequent ischemia.

Sci Sig 2010; 3: ra15

"When we see men of a contrary character, we should turn inwards and examine ourselves"

Confucius (551-479 BCE), Chinese thinker and social philosopher, whose teachings and philosophy have deeply influenced Chinese, Korean, Japanese and Vietnamese thought and life. His philosophy emphasized personal and governmental morality, correctness of social relationships, justice and sincerity.

Molecular Adsorbent Recycling System Therapy in the Treatment of Acute Valproic Acid Intoxication

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KEY WORDS: MARS (molecular adsorbent recycling system), valproic acid intoxication, hyperammonemia, hemofiltration, hemodialysis

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Acute valproic acid intoxication may be a life-threatening condition, with involvement of multiple organ systems, including the liver, kidneys, brain, heart and bone marrow. We report a case of acute valproic acid intoxication in a young man presenting with hyperammonemia and encephalopathy. MARS therapy was initiated successfully with a decrease in ammonia and valproic acid levels and resolution of the encephalopathy.

PATIENT DESCRIPTION

A 29 year old man was admitted to the emergency room due to acute overdose of valproic acid. His medical history included alcohol abuse, anxiety and depression. He was medicated with escitalopram, clonazepam and valproic acid. Several hours prior to admission he ingested 10 g of valproic acid, and was brought to the ER while suffering from abdominal pain, nausea and drowsiness. In the ER he was treated with active charcoal and fluid resuscitation. He did not require respiratory support. His blood valproic acid level at admission was 236 µg/ml (therapeutic serum concentrations range from 50 to 125 µg/ml).

He was admitted to the intensive care unit where he was treated with fluids, active charcoal and thiamine. During hospitalization he was still confused and lethargic but required neither airway protection nor respiratory or inotropic support. Blood tests showed normal liver enzyme levels and coagulation; renal function tests were also normal, as were hemoglobin, glucose, electrolytes and platelets. His blood ammonia level was 400 µg/ml on admission (normal blood ammonia level 20–85 µg/dl). An arterial blood sample demonstrated a pH of 7.34; oxygenation and ventilation were adequate, lactate level was 4.6, his anion gap was 15 and his base excess minus 4.5.

Lactulose and carnitine, 50 mg/kg, were added to his therapeutic regimen and a course of molecular adsorbent recycling system was implemented. The rationale for using MARS in our patient was that since valproic acid has a high affinity to albumin, MARS, as an albumin-based dialysis, competing with the patient's endogenous albumin for valproic acid binding, can be used in the detoxification process.

During his stay in the ICU he regained full consciousness, his blood ammonia levels dropped to 278 µg/ml on the second day and to 157 and 50 µg/ml in the following days. He was released in satisfactory condition to the internal medicine department for further observation.

COMMENT

Extracorporeal removal of valproic acid by hemodialysis and hemofiltration in acute valproic acid intoxication has been reported in the literature [1,2]. Theoretically, the relatively low molecular weight and low volume of distribution of valproic enables potential benefit from extracorporeal therapy, but the high degree of protein binding of valproic acid hinders the efficiency of this method. However, at toxic high plasma levels of valproic acid, the protein binding sites become saturated and the efficiency of extracorporeal removal of valproate increases.

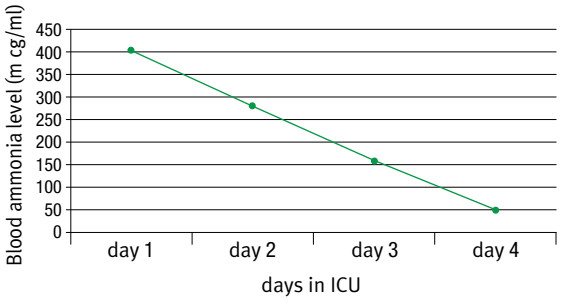
The use of a charcoal hemoperfusion cartridge may slightly increase the clearance of the drug, because there is direct contact of blood with an adsorbent that can remove even highly protein-bound drugs [3]. Hemodialysis may reverse the adverse metabolic effects of valproic acid intoxication.

To the best of our knowledge, this is the first report of MARS therapy for the treatment of acute valproic acid intoxication. MARS is an extracorporeal liver support device, usually used to provide time for hepatic regeneration or for donor availability for liver transplantation (bridge to transplantation) [4]. The principle underlying the extracorporeal liver support system is albumin dialysis, first introduced by Stange et al. [5]. This system mimics the biological feature of the hepatocyte membrane by transferring protein-bound and water-soluble toxic endogenous metabolites (such as ammonia, lactate and phenols) from the blood into a dialysate compartment through a special membrane [4]. The membrane in MARS is a highly permeable hollow fiber embedded with albumin and is used to dialyze the patient's blood against a dial-

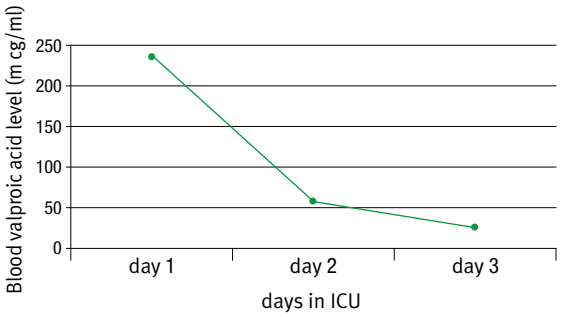
ER = emergency room

MARS = molecular adsorbent recycling system
ICU = intensive care unit

[A] Blood ammonia level during hospitalization



[B] Blood valproic acid level during hospitalization



ysis solution that contains toxin-binding carrier proteins [4].

The rationale for using MARS in our patient was that valproic acid has a high affinity to albumin, and therefore MARS can be used in the detoxification process.

Our patient did not present any signs of hepatic failure, and his encephalopathy and hyperammonemia were probably induced by the valproic acid intoxication and not by a secondary insult to the liver caused by the drug. Valproic acid may cause hyperammonemia by several mechanisms not hepatically related, which may explain the absence of elevated liver enzymes and normal coagulation factors in the presence of marked hyperammonemia. Nevertheless, the clearance of ammonia was facilitated by MARS therapy and the valproic acid levels in the patient's blood were markedly decreased once MARS therapy was initiated [Figure A].

The patient's valproic acid blood level was 236 µg/ml on admission and dropped to 59 µg/ml the following day. Two hours later the drug level was 27 µg/ml. Since the half-life of valproic acid is 7–15 hours, it is expected that when the drug level is 236 µg/ml, the elimination toward a therapeutic level will occur within 2 days. With MARS therapy, elimination of valproic acid seemed to be faster, with valproic acid levels dropping to the therapeutic range within 24 hours [Figure B]. Marked clinical improvement was demonstrated soon after MARS therapy, with the patient regaining full consciousness. This improvement may be attributed not only to valproic acid elimination via MARS therapy, but also to elimination of ammonia.

The standard care for patients with valproic acid overdose, when there is no overt liver damage or renal failure, is to observe the patient in an ICU setting. However, this patient was treated more aggressively, since despite supportive treatment consisting of fluids, active charcoal, lactulose and carnitine, no clinical improvement was noted.

In conclusion, we report the successful use of MARS therapy in a patient with acute valproic acid intoxication, hyperammonemia and encephalopathy. Further investigation is required to evaluate the benefits of MARS therapy in acute valproic acid intoxication compared to other hemodialysis and hemofiltration techniques.

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Capsule

Fibroblast growth factors and their receptors important for healthy skin

The skin acts as one of our primary defenses, protecting our organs and tissues from a dry and often hostile environment. During development, fibroblast growth factors (FGFs) and their receptors (FGFRs) help to produce and maintain a robust epidermis. Yang et al. generated mutant mice that lacked FGFR1, FGFR2, or both. Mice lacking keratinocyte FGFR1 appeared normal throughout development; those lacking keratinocyte FGFR2, however, had a reduced number of hairs and no sebaceous glands. Mice lacking both receptors displayed a more severe phenotype: As they aged, hair was lost, and the outer layer of the skin – the dermis – underwent fibrosis as a consequence of an increased inflammatory response. The tight junctions that hold skin cells together were also down-regulated in the mutant mice, which correlated with an impairment of epidermal barrier function.

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Eitan Israel

Mucosal Small Bowel Metastasis from Uterine Leiomyosarcoma

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KEY WORDS: uterine leiomyosarcoma, mucosal small bowel metastases, abnormal [^{18}F]-2-fluoro-deoxy-D-glucose uptake

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Uterine leiomyosarcoma is a mesenchymal neoplasm composed of smooth muscle. It is the most common uterine pure mesenchymal tumor, although it represents only 5% of uterine malignancies [1]. It affects 0.4/100,000 women a year [2]. Owing to the aggressive nature of this tumor, women with uterine sarcomas have a poor prognosis with an overall survival of 50% at 2 years, even when diagnosed early [1]. Surgical resection is the treatment of choice and may be curative for lesions confined to the uterus. The role of radiotherapy and chemotherapy is well established, but there is no consensus regarding the optimal management of these patients. Metastases are common and recurrence rates are high, possibly due to the presence of micrometastases at the time of diagnosis.

We present here a patient who was diagnosed with leiomyosarcoma of the uterus presenting with distant metastatic spread in a rare location.

PATIENT DESCRIPTION

A 60 year old woman was admitted to hospital in March 2009 for exploratory laparotomy due to abnormal uptake on positron emission tomography with radiolabeled [^{18}F]-2-fluoro-deoxy-D-glucose scan, revealing the presence of a small lesion suspected to be a metastatic leiomyosarcoma in the left lower abdominal quadrant.

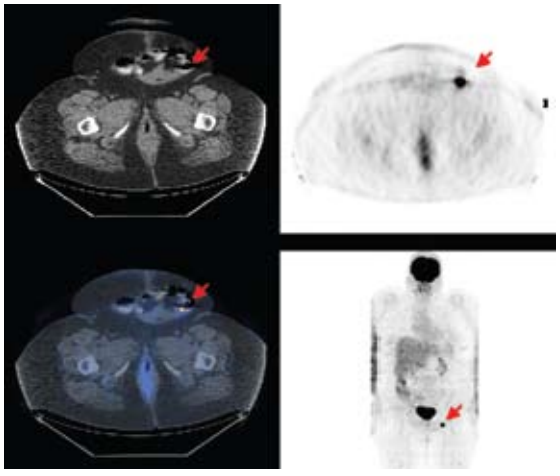
Her medical history was remarkable for recurrent metastatic uterine leiomyosarcoma that was repeatedly treated with wide local excisions. In 1993 at the age of 44 and still premenopausal, the patient was diagnosed with symptomatic uterine myomas. Due to her morbid obesity and three prior cesarean sections, follow-up only was prescribed.

In 2005, at the age of 56, she was admitted for evaluation of abdominal pain. Diagnostic workup revealed a large pelvic mass causing partial small bowel obstruction. On surgical exploration a large (24 cm) pelvic mass originating from the uterus was revealed. A total abdominal hysterectomy and bilateral salpingo-oopherectomy was performed. Neither enlarged lymph nodes nor distant metastases were evident at that time. Histopathology examination showed spindle cell leiomyosarcoma with areas of myxoid and pleomorphic cells with a mitotic index of 10–15 mitotic figures per 10 high power fields. At the same time chest computed tomography scan revealed a lesion suspected to be a metastasis in the lower lobe of the left lung. This was confirmed by PET-CT and a segmental lobar metastasectomy was performed. Histopathology revealed a metastatic lesion of the primary uterine leiomyosarcoma. A local recurrence at the same site was found and resected at the end of the same year. In 2007, a right upper lobe metastasis was discovered by routine follow-up PET-CT scan. Consequently, another segmental lobar metastasectomy was performed.

In 2008, a routine PET-CT showed increased FDG uptake in the left lobe of the thyroid suspected to be a metastatic lesion. The patient underwent a partial thyroidectomy and again histopathology revealed a metastatic lesion of leiomyosarcoma.

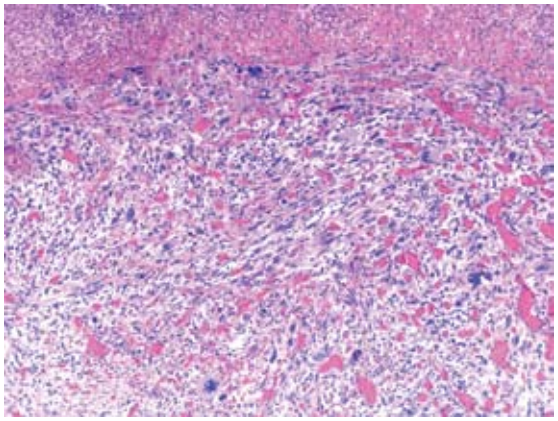
In March 2009 a follow-up PET-CT scan showed increased FDG uptake in the left lower abdominal quadrant suspected to be a small metastatic lesion [Figure A]. At laparotomy no peritoneal, hepatic, retroperitoneal or intestinal serosal spread was found. Thorough manual examination of the small bowel revealed a small (3 cm) intraluminal polypoid mass at mid-jejunum. Segmental small bowel resection was performed and the

[A] Abnormal focal FDG uptake in left lower quadrant presumed to be metastatic lesion by (transaxial) PET-CT



PET-CT = positron emission tomography-computed tomography
FDG = [^{18}F]-2-fluoro-deoxy-D-glucose

[B] Leiomyosarcoma, infiltrating mucosa of small intestine hematoxylin and eosin, x50



patient recovered uneventfully. The histopathology report confirmed that the mass was a metastatic lesion of spindle cell leiomyosarcoma with pleomorphic areas of uterine origin [Figure B].

COMMENT

Uterine sarcomas are rare tumors that account for 5% of all uterine malignancies. Mean age at presentation is 55 years [3]. Leiomyosarcomas account for 25% of uterine mesenchymal tumors, endometrial stromal sarcomas for 15% and mixed mullerian tumors for 50% of the tumors, the latter being referred to as metaplastic carcinoma (carcinosarcoma) [4]. Sarcomas most commonly invade and spread locally, but may have an aggressive growth pattern with lymphatic and hematogenous spread. Micrometastases are often present at the time of diagnosis. The most common sites for metastatic spread are the peritoneal cavity and the omentum (30–50%), lung (30–40%) and liver (10%) [3]. Metastases to the heart, pericardium, skin, stomach and pancreas have also been described.

Metastases to the gastrointestinal tract from extra-abdominal sites are uncommon. Malignant melanoma and carcinoma of the breast and lung are the most common malignancies spreading to the gastrointestinal tract.

Small bowel mucosal metastases from uterine leiomyosarcomas are extremely rare. To the best of our knowledge, only one prior case was reported in the English-language literature [5]. This rare occurrence should be suspected in patients who present with abnormal FDG uptake in the abdominal cavity and carefully searched for during exploration. Even though leiomyosarcoma is an aggressive and lethal tumor, it can be treated with routine follow-up and aggressive intervention for metastatic spread if and when it occurs.

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Capsule

Enzymes as oncometabolites contribute to the biology of brain tumors and leukemia

Identification of genes that are recurrently mutated in human tumors can potentially lead to new cancer treatments, but first we need to understand how the mutations alter the biochemical activity of the encoded protein and contribute to tumor development and progression. The recent discovery that a subset of human brain tumors harbor mutations in the gene encoding isocitrate dehydrogenase 1 (IDH1) has focused interest on this cytosolic metabolic enzyme and its mitochondrial homolog IDH2. Mutations in these genes have been detected in acute myeloid leukemia that always alter the same amino acid in the enzymes' catalytic sites and are always present in heterozygous form, suggesting that tumor cells contain "normal," as well as mutant, versions of the enzymes.

Ward et al. (*Cancer Cell* 2010; 17: 1) and Dang et al. (*Nature* 2009; 462: 739) show how the tumor-associated mutations alter the biochemical activity of IDH1 and IDH2. The mutant enzymes not only lose their normal activity (the conversion of isocitrate to α-ketoglutarate) but also acquire a new activity: the reduction of α-ketoglutarate to 2-hydroxyglutarate. Indeed, elevated levels of 2-hydroxyglutarate were detected in human tumor samples that contained either IDH1 or IGH2 mutations. Determining how 2-hydroxyglutarate, a so-called oncometabolite, contributes to the biology of brain tumors and leukemia will be an important next step in moving from mutant gene to therapy.

Eitan Israeli

"Nearly all men can stand adversity, but if you want to test a man's character, give him power"

Abraham Lincoln (1809-1865), 16th President of the United States until his assassination in April 1865. He successfully led his country through its greatest internal crisis, the American Civil War, preserving the Union and ending slavery.

Symptomatic Spinal Hemangioma in Pregnancy

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KEY WORDS: hemangioma, lumbar spine, pregnancy, vertebroplasty, preoperative embolization, anticoagulant treatment

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Hemangiomas are among the most common benign tumors involving the spine, usually presenting as an incidental finding on magnetic resonance imaging or even on plain radiography [1]. First described by Virchow in 1867, this lesion is estimated to have an overall incidence of 10–12%, as based on several cadavers and spine film reviews [2]. The lesion may involve any portion of the spinal column, but is more prevalent in the thoracic and lumbar spine segments. The mechanism of growth underlying this often slowly developing lesion is not fully understood. Pregnancy, however, is a well-documented risk factor accompanying its symptomatic conversion, often necessitating surgical decompression.

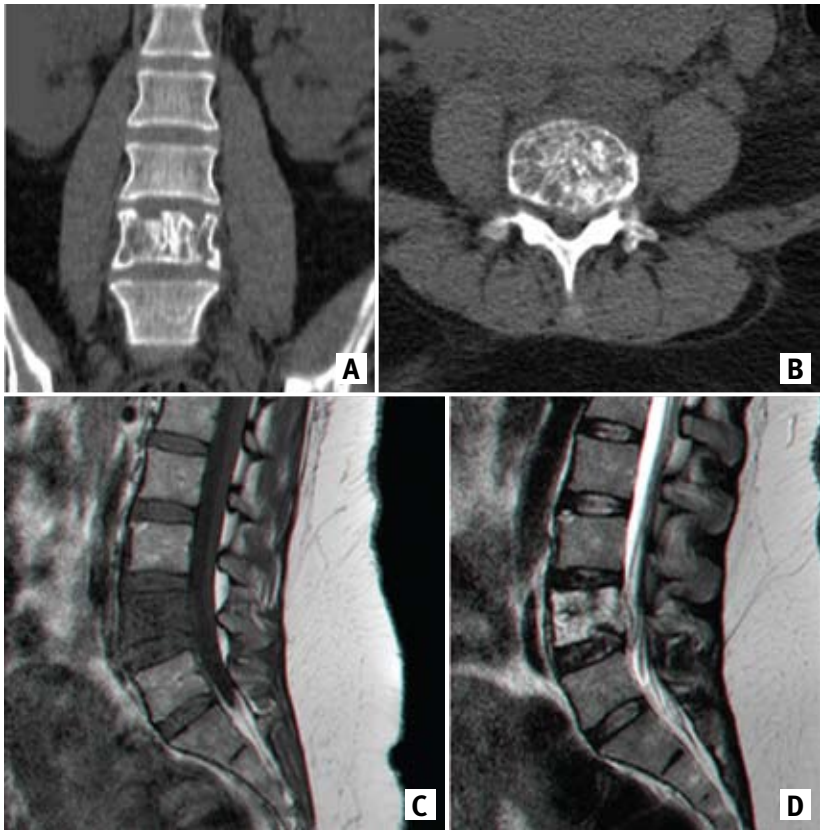
We report the case of a spinal hemangioma during pregnancy, with unusually severe intra- as well as postoperative complications.

PATIENT DESCRIPTION

A 35 year old woman presented to the emergency room in the 37th week of gestation with complaints of right abdominal and low back pain. Apart from a history of non-specific low back pain, the patient recalled that 2 weeks previously she had felt a sudden painful snapping sensation in her back that had progressively worsened and began to radiate to her left leg. Diagnosis of premature rupture of membranes led to an attempt to induce delivery. However, due to failure of both mechanical and pharmacologic means to bring on an active labor over a 48 hour period, together with the patient's overall exhausted state, a cesarean section was performed under general anesthesia.

The operative course was uneventful. Since her complaints persisted postoperatively, she was referred to an orthopedic consultation. Initial physical examination revealed diffuse tenderness in the regions of the lumbar spine and left buttock. Neurologic examination was marked by a 3/5 weakness in her left tibialis anterior muscle. Lumbar spine X-rays did not demonstrate any pathology. Computed tomography scan revealed a lesion at the fourth lumbar vertebra, marked by lytic and sclerotic areas as well as vertical striations centered in the vertebral body. Soft tissue involvement of the left lateral recess and compression of the thecal sac and the L4 nerve root were also noted [Figure]. A differential diagnosis at this point included a vertebral hemangioma

Anterior-posterior **[A]** and lateral **[B]** computed tomography radiographs; T1 **[C]** and T2 **[D]** weighted magnetic resonance images of the lumbar spine demonstrating the lesion in the L4 vertebra.



or another primary space-occupying lesion. An additional evaluation, which was carried out to rule out a possible primary malignancy, e.g. multiple myeloma or breast cancer, was negative. Magnetic resonance imaging confirmed the presence of a lesion in the L4 vertebral body, characterized by a mostly low signal intake on T1 and a high signal intake with hypo-intense striated foci on T2-weighted images [Figure].

On admission to the orthopedic department, a standard anticoagulation regimen consisting of low molecular weight heparin, 40 mg daily, was initiated since the persistence of her back pain had resulted in decreased ambulation.

Four days after admission, routine physical examination was marked by rest tachypnea resulting in respiratory decompensation. A clinical suspicion of an acute thromboembolic event was confirmed by computed tomography angiography with a demonstration of an embolus involving the right posterobasal pulmonary segment. Evidence of deep vein thrombosis on ultrasonography led to the installation of an inferior vena cava filter and initiation of full dose anticoagulation therapy.

A week after admission and after a 24 hour cessation of anticoagulation treatment, an attempted percutaneous transpedicular biopsy was performed. During the procedure, bleeding that seemed to originate from vertebral body arteries could only be controlled with hemostats. After failure to attain a biopsy, the patient was discharged and a more thorough tissue-sampling procedure was planned. An outpatient assessment of her coagulation functions did not reveal any known pathologies.

The patient was readmitted several weeks later for an open biopsy of the lesion and decompression of the painful and compressed L4 nerve root. Prior to the procedure, a percutaneous metallic coil embolization was performed by identifying and obstructing the lesion's nourishing arteries. The following day, an open laminectomy and foraminotomy at the level of L4 vertebrae enabled a broader retention of tissue sample and decompression of the compressed nerve root. An L4 vertebroplasty concluded the procedure. The postoperative course was uneventful. On discharge, the patient reported a slight improvement in the level of back pain. One year after the procedure, the patient has almost complete resolution of her leg weakness and has satisfactorily returned to her prior level of activity.

COMMENT

Hemangiomas are the most common benign vascular bone tumor and usually involve the spinal column and skull. The first known report of a spinal hemangioma in pregnancy is ascribed to Balado in 1927 [3]. In routine practice, the radiographic appearance on CT demonstrating the typical "honeycomb" pattern consisting of vertically oriented striations usually delivers the diagnosis with no need for further evaluation. Exceptions, as portrayed in the current case report, include a non-typical radiographic appearance (e.g. soft tissue involvement, pathologic fracture or compression of neural elements) and significant neurologic impairment.

Hormone regulation and hemodynamic factors are considered leading factors in the lesion's rapid growth during pregnancy. Locally, the enlarging third-trimester uterus increases the pressure in the paravertebral veins by compressing the vena cava. In addition, increased venous distension and vascular growth, thought to be mediated by the systemic influence of progesterone and estrogen respectively, might further increase local venous pressure. These two mechanisms appear to act in synergy on the lesion's close environment, adding to the probability of a compression effect on adjacent neural structures.

Symptomatic conversion leading to neurologic compromise is believed to be due to several pathologies. Direct vascular expansion that originates from the vertebral elements, mainly the vertebral body, is the most common mechanism. Other mechanisms, such as an enlarging extradural mass (e.g. bleeding) and pathologic fractures, both result in compression of neural elements.

The case presented here has several unique characteristics. Anatomically, most of the previously described symptomatic hemangiomas related to pregnancy were found to involve the thoracic, followed by cervical segments. Reports of lumbar involvement are rare, particularly of pregnancy-related symptomatic lesions.

Clinically, a life-threatening embolic event complicating a diagnosis of an osseous hemangioma is rare. Possible causes for such an event can be classified as being either primary or secondary. Although a rare coincidence of soft tissue angiomas and a consumptive coagulopathy has been described (Kasabach-Merritt syndrome), no direct evidence linking osseous hemangiomas and other clinically significant coagulopathies were found. We hypothesize that two known predisposing factors were involved in the acute embolic event: the continued immobilization imposed by the painful back pain and the general hypercoagulable state of late pregnancy and the puerperium.

Management of acute neurologically disabling lesions in the pregnant woman has been a subject of considerable debate. In addition to surgical decompression, other treatment alternatives include radiotherapy, percutaneous sclerotherapy by ethanol injection and arterial embolization, the latter being supported by a large body of evidence as an important adjunct measure in controlling local bleeding [4].

A management algorithm based on a literature review [5] highlighted two main key aspects in the decision-making process of such scenarios: the week of gestation and the severity of the neurologic impairment. Conservative management, consisting of rest and pain control, was offered to patients approaching the end of their pregnancy

(32nd week of gestation or later) or those with only mild or moderate symptoms, whereas any substantial or progressing neurologic deficit was considered an indication for surgical decompression. However, the modality preferred or its timing was not discussed.

Despite the accumulating information on the management of symptomatic hemangiomas during pregnancy, we found no class I data to support any specific modality of treatment, nor the preferred timing for its application. Recommendations for dealing with the hypercoagulable state are also lacking.

In summary, after reviewing the relevant literature and based on our limited experience, we suggest a multidisciplinary approach including interventional radiology for the management of symptomatic hemangiomas affecting the vertebral column, including those related to pregnancy. Preoperative arterial embolization should be considered as an adjunct to any therapeutic modality due to its relative high benefit-risk ratio; and given the increased risk of an acute embolic event due to the hypercoagulable state of pregnancy and the accompanying immobilization, anticoagulant therapy should be started regardless of a planned surgical procedure.

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Capsule

Thalidomide teratogenicity target and mode of action

In the late 1950s and early 1960s, thalidomide was prescribed to pregnant women as a cure for morning sickness, but it was then found to have developmental defects, most obviously, stunted limbs in thousands of babies. Although its use was banned worldwide, thalidomide has since been found to be a valuable treatment for a range of cancers, inflammatory disorders, and leprosy. Several hypotheses have been proposed, but the mechanism of action of thalidomide is unknown. Using zebrafish and chicken as animal models, Ito et al. show that the protein cereblon is a primary target of thalidomide. Thalidomide exerts teratogenic effects by binding to cereblon and inhibiting associated enzymatic activity important for limb development. Knowing the mechanism of action of thalidomide should encourage the search for thalidomide derivatives without teratogenic activity.

Science 2010; 327: 1345

Eitan Israeli

Capsule

Immunology and autoimmunity different in mice and humans

With a routine blood test, your doctor can ascertain how well your metabolism handles lipids and whether you are vulnerable to heart disease. But don't expect to get a test that reveals whether your immune system is working normally or whether you are at risk for, say, autoimmune diseases. The reason: researchers still can't define what's normal for the immune system. Cardiologists can specify healthy levels of low density lipoprotein, high density lipoprotein, and triglycerides, but immunologists can't do the same for cytokines – key chemical messengers that trigger immune cells to mature, divide, attack, or perform other actions. Researchers' reliance on mice deserves some of the blame for this ignorance, immunologists say. The human and mouse lineages diverged some 65 million years ago, and the rodent's immune system has adapted to safeguard a small, short-lived animal that scurries around with its nose in the dirt. However, nobody has cataloged the differences, and as a result, inconsistencies between human and mouse immunity often leave patients in the lurch.

Science 2010; 327: 1573

Eitan Israeli

"In the depth of winter, I finally learned that within me there lay an invincible summer"

Albert Camus (1913-1960), French Algerian author, philosopher and journalist. He was awarded the Nobel Prize for Literature in 1957 and his most famous work is the novel *L'Étranger* (The Stranger).

Overestimation by a Hand-Held Glucometer of Blood Glucose Level due to Icodextrin

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KEY WORDS: hypoglycemia, icodextrin, critical care

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It is often difficult to determine the cause of changes in mental status in critically ill patients. Clinicians often rely on data collected by various means – such as medical history, physical examination, imaging, laboratory tests – to help diagnose the condition. However, laboratory data can be misleading. Caregivers should be aware of conditions that might falsely change the results. In the following case we describe such a discrepancy in a hand-held glucometer, a widely used device that is usually accurate.

PATIENT DESCRIPTION

A 30 year old man with a history of coronary artery disease, diabetes mellitus, and end-stage renal disease treated with peritoneal dialysis presented after 2 days of hematemesis. He reported compliance with all medications, including clonidine, clopidogrel, ezetemibe, diphenhydramine, metoprolol, atorvastatin, citalopram, insulin glargine, metoclopramide, lisinopril, and omeprazole. He had also been compliant with peritoneal dialysis. He was admitted to the intensive care unit and treated with intravenous pantoprazole, intravenous hydration, and monitoring of his gastrointestinal bleeding. Following admission, repeat laboratory data showed the development of anion gap metabolic acidosis, with hyperglycemia and positive serum acetone. Diabetic ketoacidosis was diagnosed and an insulin drip initiated.

On the second hospital day there was an abrupt deterioration in his mental status. He was unresponsive to verbal stimuli and poorly responsive to physical stimuli. His pupils reacted normally to light, and there were no focal neurologic deficits. His blood pressure, heart rate, respiratory rate, and temperature were normal. His O_2 saturation was 97% while receiving oxygen via nasal prongs at 2 L/min. Cardiac, pulmonary and abdominal examinations were normal. Bedside finger stick glucose testing measured 188 mg/dl on two separate occasions.

Because of a strong concern regarding hypoglycemia, 25 ml of 50% dextrose was administered intravenously after blood had been drawn for a serum basic metabolic panel. The patient's mental status quickly returned to normal. The BMP, sent before administration of dextrose, revealed a serum glucose level of 18 mg/dl.

COMMENT

Hypoglycemia, frequently encountered in intensive care units and emergency departments, is a life-threatening yet easily treatable condition. Using hand-held glucometers generally facilitates the diagnosis of this condition, thus enabling a quick response by administering glucose. Some conditions, however, have the potential to interfere with glucose measurements by hand-held glucometers and provide incorrect values for serum glucose.

In patients with end-stage renal disease, peritoneal dialysis is used as an alternative to hemodialysis. In PD a glucose-based dialysate is used for ultrafiltration. Over time, however, structural changes in the mesothelium may lead to a reduced effect of PD [1]. Icodextrin, a cornstarch-derived glucose polymer, may be used as an alternative to glucose-based dialysates to improve ultrafiltration [2]. When used in PD fluid, 20–30% of icodextrin is absorbed into the systemic circulation and is metabolized to oligosaccharides such as maltose, maltotriose and maltotetrose [3]. Icodextrin may also be found in some chemotherapy solutions.

Icodextrin metabolites, especially maltose, may be detected with some glucometer enzymatic reactions and falsely measured as glucose [2-5]. Most glucometers use one of two enzymes – glucose oxidase or glucose dehydrogenase – to detect the presence of glucose. In these reactions, glucose is metabolized by GOD or by GDH to hydrogen peroxide or reduced nicotinamide adenine dinucleotide, respectively. The amount of hydrogen peroxide or rNAD can then be measured by oxidized dye color change or by electrochemical reactions to calculate the amount of glucose present [1]. Reducing agents other than glucose, such as metabolites of icodextrin, may also be detected by these methods. Since neuronal cells use glucose as the primary source of adenosine triphosphate

BMP = basic metabolic panel

PD = peritoneal dialysis
GOD = glucose oxidase
GDH = glucose dehydrogenase
rNAD = reduced nicotinamide adenine dinucleotide

production and poorly utilize maltose as an energy source, the false detection of maltose or maltotriose as glucose can obviously have dire consequences if serum glucose levels are in fact very low.

While it is generally thought that the GDH method is more susceptible to false detection of glucose by icodextrin metabolites [2,3], both methods are susceptible to interference [4]. One study showed that in 25 patients treated with icodextrin-containing dialysate fluid [5], using a GOD-based glucometer, glucose level measurements were elevated by more than 20% in all but one measurement, when compared with those measured by a laboratory-based system.

Other conditions also have the potential to interfere with glucose measurements by hand-held glucometers. High or low hematocrit values can lead to falsely abnormal glucose readings. Also, high levels of uric acid, which is a reducing agent, may result in falsely high glucose readings [1]. Both of these conditions are common in critically ill patients and in patients with renal failure. The table describes how the presence of icodextrin and its metabolites, high uric acid levels and changes in hematocrit interfere with the measurement results of different types of glucometers. The information was compiled from data published in the literature [4] and warnings published by the manufacturers of the devices and the manufacturer of a peritoneal dialysate containing icodextrin (Extraneal® by Baxter).

Table. Interference in results of glucose measurements with icodextrin, uric acid and high or low hematocrit

Device name and manufacturer	Icodextrin	Uric acid	Hematocrit
Glucotrend®, Roche Diagnostics	Yes	No	No
Advantage®, Roche Diagnostics	Yes	Yes	Yes
Accu-Check®, Roche Diagnostics	Yes	No	Yes
MediSenseG2®, MediSense	No	No	Yes
One Touch®, Lifescan	No	No	Yes
FreeStyle Systems®, CoZmonitor® and Optium Omega®, Abbott	Yes	No	Yes
Ascensia® (except for Ascensia Contour GDH-PQQ), Bayer	No	N/A	N/A

Data from the literature [4] were provided by manufacturers of the devices and the manufacturer of icodextrin (Baxter). For certain devices the some information was not available (N/A).

In our patient, the low glucose measured by the serum basic metabolic profile clearly indicated true hypoglycemia, and administration of intravenous glucose abruptly reversed his mental status change. The glucose level of 188 mg/dl on the hand-held glucometer indicates substantial interference of the glucose measurement by metabolites of icodextrin. As strict glycemic control becomes ever more common in critical care settings, accurate glucose measurements are essential.

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Capsule

Automated method for tracking the migration of circulating tumor cells

Certain cancers spread through the migration of circulating tumor cells (CTCs). Once shed from either primary or metastatic sources into the bloodstream, these cells can become lodged in bone, lungs, brain or liver. The rarity of the cells, at concentrations of one per billion blood cells, has hindered their use in quantitative evaluations. Stott et al. have developed an automated imaging system for prostate cancer CTCs. The cells are isolated with a micro-fluidic chip that extracts them from the leukocytes and red blood cells. They are then stained to highlight the nuclei and prostrate-specific antigens (PSAs), as the distance between the two markers can be used to identify and verify each whole cell. Significant PSA heterogeneity was detected across the CTCs taken from a range of patients, and there was also considerable variability in the rate of decline of CTCs after surgery. However, the authors were able to track significant decreases in CTCs in patients with metastatic cancer after hormone therapy, with only modest decreases in CTCs after chemotherapy. The authors envision scaling up this automated method for tracking the migration of CTCs.

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Eitan Israeli

Should We Operate on Occult Hip Fractures?

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KEY WORDS: occult fracture, hip fracture, subcapital hip fracture, intertrochanteric fracture

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Occult hip fractures are well described in the literature, with the incidence of radiographically occult fracture estimated at 2–9% [1]. When an elderly osteoporotic patient presents a typical history and clinical presentation of a fall and hip pain, plain radiograph should confirm the diagnosis. When the X-ray is interpreted as normal, an occult fracture should be suspected and the patient should undergo investigation. Several modalities have been proposed such as computed tomography, bone scan and magnetic resonance imaging, which is now the study of choice [2-5]. The treatment for the occult fracture is not discussed in the literature and patients undergo conservative or surgical treatment. In this article we describe two cases of occult hip fracture, review the literature regarding the treatment of this kind of fracture and discuss the optional treatments.

PATIENT DESCRIPTIONS

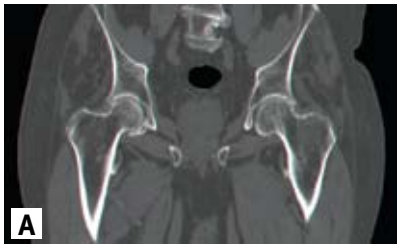
PATIENT 1

An 87 year old man presented to the emergency room with hip pain following a fall. He was discharged after plain X-ray failed to demonstrate a fracture. Two weeks later he returned to the ER because of continued pain and ambulation only with assistance.

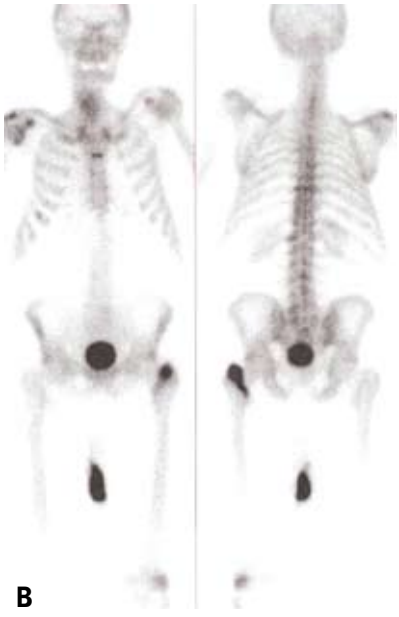
ER = emergency room

CT scan was performed [Figure A] and was interpreted as negative for fracture. He was admitted and a Tc99 bone scan was performed showing uptake in the intertrochanteric region [Figure B]. We discussed the treatment options and decided that since the patient was able to ambulate we would not operate. The patient's follow-up was unremarkable and he was able to walk unassisted.

[A] CT coronal view of pelvis. The CT does not demonstrate a fracture



[B] Tc99 bone scan demonstrating uptake in the intertrochanteric region



PATIENT 2

An 82 year old male presented to the ER with hip pain following a fall the day before. Plain X-ray demonstrated a fracture of the greater trochanter, with CT scan demonstrating the same fracture. He was admitted and a Tc99 bone scan was performed that showed uptake in the intertrochanteric region. The patient was able to move his leg and to sit. Non-operative treatment was agreed upon, and partial weight bearing was obtained one week later. After another week the patient was walking with minimal pain and at 2 months follow-up he was ambulating with no aid.

LITERATURE REVIEW

All the articles reporting occult hip fracture discuss the diagnosis strategy but not the treatment options. We found only six articles describing the treatment. Pandey and colleagues [3] describe 19 occult hip fractures (14 subcapital and 5 intertrochanteric); surgery was performed on all the patients except for one with an intertrochanteric fracture due to coexisting medical problems. Quinn et al. [2] report 11 occult hip fractures (5 subcapital and 6 intertrochanteric) and all their patients underwent surgery except for one with an intertrochanteric fracture that was inoperable because he was not suitable surgically. Rubin and team [4] describe 12 occult hip fractures (5 subcapital and 7 intertrochanteric), and all the patients underwent surgery except for one with an intertrochanteric fracture. In the series of 25 occult hip fractures (11 subcapital and 14 intertrochanteric) reported by Rizzo et al. [1], all the patients underwent surgery except for 4 with intertrochanteric fractures (the

reason was not stated by the author) and managed with partial weight bearing. Alba and co-authors [5] reported four neck fractures; one patient was treated non-operatively with bed rest.

COMMENT

Occult hip fractures are common and the treating physician should suspect this type of fracture and be aware of the modalities to identify this fracture. The literature does not discuss the treatment strategy for occult hip fractures, and surgery seems to be the treatment of choice, as for other usual hip fractures, in order to reduce morbidity and mortality. We believe that occult hip fractures should be divided into two groups: cervical and intertrochanteric.

The occult cervical fractures are non-displaced intraarticular fractures that can easily be displaced. The displacement of a cervical hip fracture raises the prevalence of non-union and avascular necrosis necessitating different treatment (hemiarthroplasty or pinning). This makes surgical treatment of the cervical hip fracture the treatment of choice. Our literature review revealed that surgery was performed in 97.4% (38/39) of cases with this kind of fracture.

The occult intertrochanteric fracture is a non-displaced fracture that is usually more stable than occult subcapital fracture and displacement does not change the surgical procedure. We only found a few authors who did not operate on this type of fracture, mainly due to medical problems. Rizzo et al. [1] was the only author who described partial weight bearing for these patients.

The goal of hip fracture treatment in the adult patient is early mobilization. We suggest the option of conservative treatment for patients with occult femoral intertrochanteric fractures who can be ambulated despite the fracture. By so doing the risk of surgical complications is obviated and also prevents complications in a non-ambulated patient.

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Capsule

New genetic associations detected in a host response study to hepatitis B vaccine

The immune response to hepatitis B vaccination differs greatly among individuals, with 5–10% of healthy people failing to produce protective levels of antibodies. Several factors have been implicated in determining this response, chiefly individual genetic variation and age. Aiming to identify genes involved in the response to hepatitis B vaccination, a two-stage investigation of 6091 single-nucleotide polymorphisms (SNPs) in 914 immune genes was performed in an Indonesian cohort of 981 individuals showing normal levels of anti-HBs versus 665 individuals displaying undetectable levels of anti-HBs 18 months after initial dose of the vaccine. Of 275 SNPs identified in the first stage (476 normal/372 non-responders) with $P < 0.05$, significant associations were replicated for 25 polymorphisms in 15 genes (503 normal/295 non-responders). Davila and co-workers have validated previous findings [*HLA-DRA*, rs5000563, P value combined = 5.57×10^{-10}; OR (95%CI) = 0.61 (0.52–0.71)]. In addition, the researchers detected a new association outside of the human leukocyte antigen loci region that passed correction for multiple testing. This SNP is in the 3′ downstream region of *FOXP*1, a transcription factor involved in B cell development [P value combined = 9.2×10^{-6}; OR (95%CI)=1.38 (1.2–1.6)].These findings might help us understand the biological reasons behind vaccine failure and other aspects of variation in the immune responses of healthy individuals.

Genes Immunity 2010; 11: 232

Eitan Israel

"An eye for an eye would make the whole world blind"

Mahatma Gandhi (1869-1948), political and spiritual leader of India during the Indian independence movement. He was the pioneer of resistance to tyranny through mass civil disobedience, a philosophyfirmly founded on total nonviolence – which led India to independence and inspired movements for civil rights and freedom across the world.

Metastasis of Cervical Carcinoma to the Distal Biliary System

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KEY WORDS: cervical cancer, metastases, biliary system

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Squamous cell carcinoma of the cervix is the most common form of cervical neoplasm. The more advanced the staging of SCCC at diagnosis, the higher the recurrence rate, which ranges from 10% to 70%. Most of the recurrences occur within 2 years after the initial treatment [1].

Blood-borne metastases are typically a late manifestation of the disease and may disseminate to almost any tissue of the body [2]. The common destinations of metastasis are the lung, bone, paraaortic nodes, abdominal cavity and supraclavicular nodes [1]. In a study of 278 autopsied cases of SCCC of various stages, the researchers assessed the influence of primary treatment on the incidence and distribution of recurrent disease and found 3.2% recurrence in the biliary system [3].

One-fifth of malignant biliary obstructions is a result of distant primary malignancies which metastasize to the pancreaticobiliary system [4]. Biliary stasis causing jaundice can occur due to obstruction of the small intrahepatic biliary ducts, parenchymal infiltration of the tumor cells, or compression of the larger extrahepatic ducts.

We report here a rare case of recurrent SCCC metastasizing to the ampulla of Vater, causing obstructive jaundice as a presenting symptom.

PATIENT DESCRIPTION

An 81 year old woman presented to our institution in May 2005 with postmenopausal bleeding. Vaginal examination revealed a 4 cm tumor in the uterine cervix spreading to the anterior wall of the vagina. A biopsy from the uterine cervix demonstrated high grade, poorly differentiated non-keratinizing squamous cell solid carcinoma.

A computed tomography scan of the abdomen and pelvis, and further evaluation with positron emission tomography-CT identified local disease in the mid-pelvic area, with no evidence of metastasis. A clinical and radiographic evaluation of the patient demonstrated a stage IIA tumor as defined by the International Federation of Gynecology and Obstetrics. Radiotherapy treatment combined with cisplatinum chemotherapy was given. The treatment was completed by December 2005. From April 2006 to September 2008, the patient was under routine periodic surveillance consisting of physical examination, ultrasound and PET-CT, with no evidence of disease.

In December 2008 the patient presented with jaundice and dark urine. Physical examination revealed a jaundiced woman with no abdominal tenderness. Laboratory data included serum total bilirubin levels of 6.2 mg/dl, direct bilirubin 3.3 mg/dl and alkaline phosphatase 403 U/L, gamma-glutamyltransferase 821 U/L. An abdominal ultrasound demonstrated dilation of the extra- and intrahepatic bile ducts. The common bile duct was dilated up to its distal end, with no evidence of thickening.

The patient was diagnosed with clinical cholangitis and antibiotic treatment was initiated, followed by urgent papillotomy by endoscopic retrograde cholangiopancreatography. During the procedure purulent bile was drained, and because of the irregular and bulgy appearance of the papilla a biopsy was taken.The patient's clinical condition and her laboratory measurements normalized soon after the procedure. A CT scan of the abdomen and pelvis showed no evidence of a local mass. She was discharged with instructions for further evaluation by endoscopic ultrasound and duodenoscopy.

There were no unusual findings in the biliary duct on endoscopic ultrasound in January 2009 and the pancreatic tissue was normal. Duodenoscopy was performed soon after and a biopsy was taken from the ampulla of Vater. The biopsy showed infiltration of the papilla by squamous cell carcinoma [Figure].

PET-CT 2 months later (March 2009) demonstrated two new hypermetabolic lesions. The first was located in the epigastrium between the head of the pancreas and the duodenum, and the other was situated near the hilum of the right lung. The patient started chemotherapy treatment for disseminated recurrent cervical cancer.

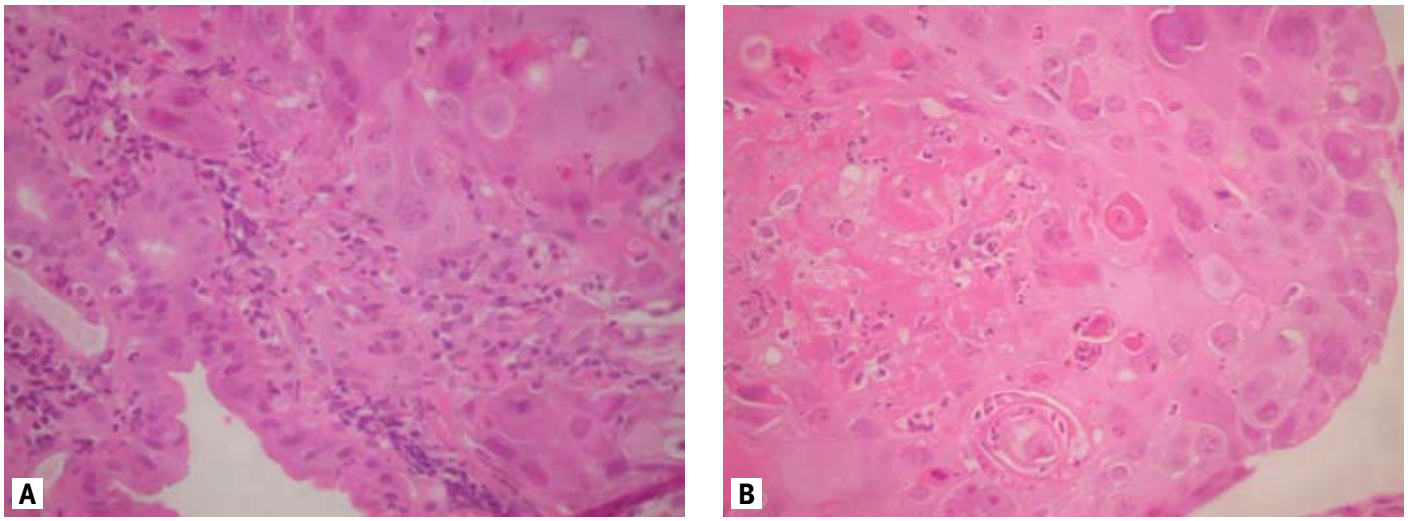
COMMENT

SCCC metastasis to the hepatobiliary tree is rare, with only five documented

SCCC = squamous cell carcinoma of the cervix

PET = positron emission tomography

Histology sections of the biopsy taken from the papilla of Vater. **[A]** Section showing normal columnar intestinal epithelial cells at the lower left margin with squamous tumor cells with large nuclei in the right upper part. This type of infiltration to the submucusal layer is consistent with lymphatic or hematogenic spreading. **[B]** Section of the metastatic tumor. At the center of the specimen is an eosonophilic keratinized cell; this is a pathognomonic finding of SCC.



cases in the literature of obstructive jaundice [5]. In most of them the biliary strictures were located proximally, in the porta hepatic area. One of the patients presented with a distal obstruction of the common bile duct by a nearby malignant lymph node [5]. That was the only reported case, besides our own, in which the patient presented with ascending cholangitis.

This is the first reported case of SCCC metastases to the ampulla of Vater and the sixth case report of jaundice as the presenting symptom for recurrent SCCC. Comparison of this case to the previously published five known cases emphasizes several features. Our patient was older than the previously reported patients (age range 38–72 years). In those cases the time between the initial treatment and the appearance of jaundice was 10 days to 32 months [5]. In our case the time interval was considerably longer, 42 months. Radiographic evidence of paraaortic lymph node involvement was observed in two of the five reported patients. In our case there was no evidence of paraaortic lymph node involvement according to the imaging studies (PET-CT).

Our case was a clear example of metastatic SCCC; we contend that in any case of a solitary squamous cell carcinoma in the biliary system, primary squamous cell carcinoma of the biliary tree should be considered. This is a rare tumor, with only five published cases, three of which involved the ampulla of Vater. Little is known about the preceding etiology; however, most reported cases have been associated with chronic biliary inflammation.

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"Reading, after a certain age, diverts the mind too much from its creative pursuits. Any man who reads too much and uses his own brain too little falls into lazy habits of thinking"

Albert Einstein (1879-1955)

"Probable impossibilities are to be preferred to improbable possibilities"

Aristotle (384-322 BC), Greek philosopher, a student of Plato and teacher of Alexander the Great. His writings cover many subjects, including physics, metaphysics, poetry, theater, music, logic, rhetoric, politics, government, ethics, biology and zoology. Together with Plato and Socrates (Plato's teacher), Aristotle is one of the most important founding figures in Western philosophy.

ONE MAN'S OPINION

To the Editor:

I would like to share with you and the readers of your journal my observations regarding the care of diabetic wounds. In Israel there is presently a very large 'diabetic population', numbering over one million persons. A significant percentage of these people suffer from a variety of complications, the most serious of which are diabetic neuropathy and peripheral vascular disease (PVD). These complications in turn lead to a large number of wounds in the lower extremities, especially the feet, in many cases resulting in amputations.

Since mine is a private clinic, I usually see patients after they have been through an extensive period of therapy in the clinics of the various health funds (*Kupot Holim*). The reason they finally turn to a private physician is that the wounds simply don't heal; in most instances their condition worsens. Several factors, in my opinion, contribute to this phenomenon:

1. In most of the cases, patients are given, as a standard, a prescription for antibiotics without the benefit of microbiologic studies.
2. Insufficient attention is paid to the proper maintenance of blood sugar levels – most of the patients I see in my practice have HbA1C levels of 8 and higher.
3. No proper correlation is attributed between antihypertensive therapy and PVD, with blood pressure lowered too much, essentially worsening the blood flow to the extremities in patients with PVD.
4. In my clinical experience, extensive use of polidine on all diabetic wounds results in the development of gangrene in the affected parts in about 80% of the cases.

I write this letter in the hope of raising awareness among treating physicians to the severity of the problems in the diabetic foot and the present inadequacy of therapies given to most patients in Israel. I would like to propose the formation of a committee/study group that would conduct an extensive comparative study of all existing therapies used throughout the country, which would then lead to the publishing of guidelines for standardized diabetic wound care in Israel. I realize that this subject has been investigated in the past and that it is an ongoing process. In my experience, however, due to the limitations of the health funds, no attempts have been made to broaden the conventional treatment to utilize the newest technology.

I hope that publication of this letter will result in stimulating a productive discussion among professionals.

Josef Strazynski MD
Vascular Treatment Center, Tel Aviv
(Clinical Associate Professor of Family Medicine, USA)

Capsule

Specific gene expression signature associated with development of autoimmune type 1 diabetes using whole-blood microarray analysis

Understanding the pathogenesis of type 1 diabetes (T1D) is hindered in humans by the long autoimmune process occurring before clinical onset and by the difficulty studying the pancreas directly. Alternatively, exploring body fluids and particularly peripheral blood can provide some insights. Indeed, circulating cells can function as 'sentinels', with subtle changes in gene expression occurring in association with disease. Reynier et al. investigated the gene expression profiles of circulating blood cells using Affymetrix microarrays. Whole-blood samples from 20 first-degree relatives of T1D children with autoimmune diabetes-related antibodies, 19 children immediately after the onset of clinical T1D and 20 age and sex-matched healthy controls were collected in PAXgene tubes. A global gene expression analysis with the MDS approach allowed the discrimination of prediabetic subjects, diabetic patients and healthy controls. Univariate statistical analysis highlighted 107 distinct genes differently expressed between these three groups. Two major gene expression profiles were characterized, including type I interferon-regulated genes and genes associated with biosynthesis and oxidative phosphorylation. These results show the presence of early functional modifications associated with T1D, which could help us understand the disease and suggest possible avenues for therapeutic interventions.

Genes Immunity 2010; 11: 269

Eitan Israel

"A life spent making mistakes is not only more honorable, but more useful than a life spent doing nothing"

George Bernard Shaw (1856-1950), Irish playwright whose works deal sternly with prevailing social problems, but have a vein of comedy to make their stark themes more palatable. Shaw examined education, marriage, religion, government, health care and class privilege.