Child Abuse and Neglect: Reporting by Health Professionals and their Need for Training

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ABSTRACT: **Background:** For health professionals who interact professionally with children, adequate awareness and training regarding the clinical indicators of child abuse and neglect, as well as subsequent reporting and procedures, are essential.

Objectives: To study Israeli health professionals' experiences with identification and reporting of suspected cases of child abuse and neglect, and their perceived training needs in this area.

Methods: The study group was a convenience sample comprising 95 Israeli health professionals (physicians, nurses, social workers, psychologists, etc.) attending workshops on medical aspects at a national conference on child abuse and neglect. In this cross-sectional survey, the health professionals were asked to complete an anonymous structured questionnaire on their experience with child abuse and neglect and on their training needs.

Results: The participants in the survey had relatively high levels of involvement with child protection. Nevertheless, they strongly expressed their need for training, especially in mastering practice skills. The need for training was greater for professionals with less experience in child protection, and there were different needs according to profession.

Conclusions: Despite their prior extensive experience in dealing with child abuse and neglect, most of the health professionals participating in the conference reported the need for training in certain areas.

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KEY WORDS: child abuse, child neglect, maltreatment, health professionals, training needs

In recent years, domestic violence in general, and child abuse and neglect in particular, have increasingly gained public attention, either because societies have become more violent or because professional and public awareness of the problem has grown. Child maltreatment is defined as a condition in which the child is either abused (commission) or neglected (omission) by adults responsible for their care. There are four major types of child maltreatment: neglect, physical abuse, emotional neglect/abuse, and sexual abuse. In 2008 there were 34,000 new referrals of maltreated children to protective services in Israel (about 16 to every 1000 children) [1].

Health professionals are often called upon to deal with cases of suspected child abuse and neglect [2]. It is essential, therefore, that they are adequately trained to recognize the clinical indicators of child abuse and neglect and to carry out the reporting requirements and procedures [3].

Physicians, and particularly pediatricians, are often the first professionals to be confronted with cases of suspected child abuse and neglect. In many cases, pediatricians become more involved when the suspicions they raise are verified. In such cases, they then work with families, child welfare agencies, police and lawyers, and are asked to provide testimony in court. When screening, diagnosing and reporting such cases, pediatricians face a complicated task due to multiple medical as well as social and contextual factors involved in this professional process. Previous studies have shown that they feel ill prepared to deal with such cases [3,4]. This low level of subjective preparedness may be one of the reasons for many unreported cases of suspected child abuse. For instance, in the Swedish study of Borres and Haäg [5] two-thirds of the interviewed physicians reported that there were incidences in which they suspected abuse but preferred not to report it. The reasons physicians avoid reporting are multifaceted [6], and include: a) the inherent difficulties in defining child abuse and inadequate training to diagnose such cases; b) fear of alienating or stigmatizing the family; c) lack of confidence in child protection services and the police; d) personal, legal and financial risks associated with reporting; and e) discomfort in taking on the role of a "policeman" [4,7-9].

The most significant factor in this context is insufficient awareness of child protection issues, which is due to inadequate training and lack of professional education on domestic violence in medical schools and residency curricula. Previous studies have identified a very low level of domestic violence

detection in pediatric practice compared with primary care settings [5,7]. Canadian psychiatry residents as well as American pediatric residents and pediatric emergency fellows all reported feeling inadequately trained for approaching, evaluating and managing child protection cases [10-12]. Inadequate knowledge on the part of physicians has also been documented [13,14]. Therefore, training is an essential component of improving physicians' skills in child protection processes [2,15,16]. Several studies have examined how health professionals who have regular professional contact with children and their families in a variety of front-line specialties assess their training in child abuse and neglect. These surveys included primary care physicians [2,3], as well as residents in pediatrics [17], accident and emergency medicine [11], and psychiatry [10]. Overall, these studies conclude that a large proportion of pediatricians perceive their training to be unsatisfactory in both intensity and focus. In particular, many are confused regarding their specific role in the child protection process, are uncertain as to what they should do and report when they come across suspected cases of maltreatment, and are especially uncertain regarding the consequences (positive or otherwise) of involving outside agencies [7]. Borres and Hägg [5] found that 60% of the respondents reported their need for training in identifying child abuse, 49% of them in recognizing symptoms, and 37% regarding the effects of abuse and neglect. The most prominent rationale for the need for training was the fact that nursing or medical education had failed to adequately cover child abuse and neglect issues. Local and regional surveys of primary care practitioners noted that their prior education and experience with child protection services, along with the characteristics of practice setting (e.g., hospital versus community based), appear to influence their decision to report suspected abuse [8,18-22]. Following these findings, several educational programs on child abuse and neglect were instituted. Bar-on [23] found that the pediatricians participating in the program were more capable of distinguishing abuse from accident cases both theoretically and in practical situations. Furthermore, they had the required skills to approach the children and their parents, and to function as members in multidisciplinary teams more effectively than those who had not participated in the program. This and other studies [8,10,12] provide support for the importance of designing training programs to improve the ability of health professionals to identify cases of suspected abuse, report them, and work effectively with the child protection system to prevent and treat such cases.

In order to tailor training programs to the specific needs of Israeli health professionals it is essential to identify their training needs in this area. The present study assesses Israeli health professionals' experiences with identifying and reporting suspected cases of child abuse and neglect, and their perceived training needs in this area.

SUBJECTS AND METHODS

The current study was based on a convenience sample of 95 Israeli health professionals attending workshops on medical aspects of child abuse and neglect, conducted as part of a national conference on the subject. The sample comprised 90.8% females; most participants were 36–55 years old (70.6%), 15.3% were older than 56, and the rest (14.2%) were younger than 35. Close to half (46.5%) of the participants were physicians, 29.1% were nurses, 15.1% social workers, 5.8% were psychologists in the health system, and 3.5% defined their profession as "other." Most of the health professionals reported that they worked in the community (53.0%) and in hospitals (41.0%). Approximately 42% of the participants had more than 21 years of experience, 26.2% had between 11 and 20 years, 20.2% had 5–10 years, and approximately 20% had less than 5 years of experience in their profession.

PROCEDURE

Information was collected from the health professionals by an anonymous structured questionnaire that was distributed to all participants in several workshops during a conference on medical aspects of child abuse and neglect. Participants were given time to complete the questionnaire before the workshops started. The response rate was approximately 95%. Anonymity was ensured, and the researchers made it clear that participants could withdraw from the study at any time for any reason.

MEASUREMENT

A self-report questionnaire was used for the study. The questionnaire included background information (age, gender, profession) as well as several scales regarding the health professionals' experience of reporting suspected cases of child abuse and neglect, and their training needs. The instrument was developed by the authors based on the relevant literature [4,7,24,25] on health professionals' training needs and reporting of child abuse and neglect. No psychometric information was available, except for internal consistencies of a training needs scale, reported below.

STUDY VARIABLES

The variables included gender, age (≤ 25, 26–35, 36–45, 46–55, ≥ 56 years old), profession (physicians, nurses, social workers, psychologists, "other"), workplace (community, hospital, "other"), and years of experience in their profession (≤ 5 years, 6–10, 11–20, ≥ 20).

We examined six types of specific experiences in identifying, reporting and taking part in professional discussions of cases of child abuse and neglect during the last year, such as "You reported a suspected case of child abuse and neglect to the police" and "You testified in court on suspected child

abuse and neglect." An "experience" scale was created by counting the types of activities in which they were involved (0 = did not happen at all in the last year) to 6 (six different types of experiences related to child abuse and neglect in the last year).

The health professionals were asked to report their level of agreement with a series of questions aimed to measure their training needs in identifying, understanding, reporting, and treating cases of child abuse and neglect. Each scale ranged from 1 (not at all) to 5 (very much). The five areas were:

- *Theoretical knowledge needs*: measured by four items (α = 0.84), e.g., "I need theoretical knowledge about the consequences of child abuse and neglect on the maltreated child"
- *Knowledge on the role and work procedures of child welfare professionals* (i.e., child investigators, child protection officers, the police, hospital-based violence committee): measured by four items (α = 0.91). It includes items such as "I need to learn the roles and work procedures of the police in cases of suspected child abuse and neglect"
- *Professional skills to accurately identify maltreated children*: measured by four items (α = 0.93), each referring to a different type of child abuse and neglect (physical, sexual, emotional, neglect). It includes items such as "I need professional skills to correctly identify children who were sexually abused in their home"
- *Skills to deal with the maltreated child and the parents:* assessed by five items (α = 0.89), including items regarding ways to cope with parents who are suspected of being abusive and the suspected maltreated child, e.g., "I need professional knowledge and skills enabling me to cope with a maltreated child's responses during the child's examination"
- *Knowledge and skills to help monitor and follow-up cases*: measured by four items (α = 0.77), e.g., "I need professional knowledge and skills on how to prepare a professional case review of suspected maltreatment, for example for the court."

RESULTS

EXPERIENCE WITH CASES OF CHILD ABUSE AND NEGLECT OVER THE LAST YEAR

Overall, many of the participants reported having experiences in the last year that involved suspected abuse or neglect: 78.9% had at least one case, 65.3% participated in a case conference on such a case, 71.6% reported at least one case to the child protective services and 29.5% reported to the police. About 45% prepared a professional report on a child abuse case and 12.6% testified in court. Overall, the health professionals reported an average of three specific experiences of identifying, reporting and participating in professional discussions of child abuse and neglect during the previous year (mean 3.03, SD 1.75, median 3.00). The minimal number of such experiences found in the current sample was 0 (10.5% of the sample) and the maximum 6 (9.5%).

PREVIOUS TRAINING

Most of the respondents had participated previously in a workshop or conference related to the issue of child abuse and neglect (86.2%), about half of the physicians during their training for their medical specialty (47.4%), and about 40% of the health professionals reported that they had had lectures on this topic when in medical school or university. Only 23.9% participating in the study said that they received intensive training as part of specialization in this area.

TRAINING NEEDS

The respondents reported on 26 specific training needs in five areas [Table 1]. Several training needs were identified by more than 70% of the respondents: skills in how to testify in court (78.2% acknowledged this need to a large or very large extent), how to talk to a child suspected of being a victim of maltreatment (74.4%), how to address a child's reactions during the examination (72.2%), and how to deal with parents who react strongly to the investigation (71.9%). About 70% of the respondents also expressed a need for skills to work in an interdisciplinary team. An overview of the five areas of training needs indicates that the need for practical knowledge and skills to identify various types of maltreatment (mean 3.75, SD 1.04) and to interact with children and parents (mean 3.66, SD = 0.89) was higher than the need for theoretical knowledge (mean 3.56, SD 0.91), monitoring and follow-up (mean 3.45, SD 1.00), and information about how other components of the protective system operate (mean 3.37, SD 1.17).

WHO NEEDS MORE TRAINING?

We examined the characteristics of respondents who expressed a need for further training. In terms of background variables, the need for training was not significantly associated with gender, age or years of professional experience. Further, there was no difference between professionals working in hospitals and those working elsewhere. However, respondents working in hospitals expressed more training needs in the area of theoretical knowledge (mean 3.82, SD 0.91), compared with others [mean 3.39, SD 0.82, t (76) = 2.20, $P < 0.05$]. When comparing physicians, nurses and others, we found that there were significant differences in their overall need for training [$F(3,80) = 3.30$, $P < 0.05$]. A more detailed analysis indicated that the sources of this effect were: significant differences in needs with regard to learning about the role of other partners in child-protective work [$F(3,80) = 7.87$, $P < 0.001$], and knowledge and skills to monitor and follow up [$F(3,80) = 4.78$, $P < 0.01$]. Schefe post-hoc tests

indicate that nurses have a greater need (compared with social workers and psychologists) to learn about the work and roles of other professions, and physicians have greater needs (compared with social workers) to train on issues of monitoring and follow-up.

The findings showed consistently that training needs were significantly higher for the professionals who had fewer experiences in dealing with maltreatment cases. Thus, previous experience was negatively associated with overall training needs (r = -0.384, P < 0.001), and with specific training needs such as knowledge on the role and work procedures of child welfare professionals (r = -0.354, P < 0.001), theoretical knowledge regarding child abuse and neglect (r = -0.241, P < 0.05), knowing how to cope with the maltreated child and the parents (r = -0.268, P < 0.01), skills to identify maltreated children (r = -0.269, P < 0.01), and knowledge and skills for reporting and monitoring suspected cases of child abuse and neglect (r = -0.346, P < 0.001).

DISCUSSION

The survey was conducted during workshops on child abuse and neglect designed for health professionals. One may assume that these workshops probably attracted those professionals who are more involved in this area. This may explain the relatively high levels of experience of the present sample with child maltreatment cases. In fact, almost 80% had experienced at least one case of suspected child abuse and neglect during their work and two-thirds had participated in a case conference on such a case. Interestingly however, despite this prior extensive experience, most of the participants in this study reported training needs in many areas. It is reasonable to assume that the training needs of health professionals who have less experience would have been even higher. The most common needs were for skills regarding testifying in court, talking with children in order to examine suspicion for maltreatment, and addressing their and their parents' reactions during the examination. These findings indicate that many of the training needs are related to practical skills – "how to" talk to children, and to their parents, "how to" prepare for court testimony, and "how to" identify signs of abuse, rather than a need for more theoretical and abstract knowledge. This trend has implications not only with regard to the training content but also for training methods. Indeed, recent training programs in this area emphasize hands-on experience and active simulations designed to provide realistic experiences under controlled circumstances that allow for multiple opportunities for feedback and self-reflection [4].

The present study found large variations among the respondents in their training needs. Hence, a "one size" training program will not fit all, and a more sophisticated approach is required to tailor efficient training programs that are appropriate for each of the subgroups. For instance, there are indications that in certain areas nurses differ from physicians in their training needs and in their experience with reporting child maltreatment, and they in turn differ from social workers. In addition, hospital-based professionals may have different training needs than community-based teams. Training programs should reflect this variability. The factor that seems most relevant to differences in training needs is

Table 1. Training needs reported by study participants regarding child abuse and neglect (N = 85–90)

% needing training*		Mean**	SD
1. Need theoretical knowledge regarding		3.56	0.91
Understanding maltreating family systems	64.0	3.76	1.13
Differential diagnosis	61.2	3.74	1.15
Laws and regulations	59.0	3.57	1.26
Causes for maltreatment	44.3	3.32	1.16
Effects of maltreatment on the child	44.2	3.42	1.07
2. Need to learn about the role and work procedures of		3.37	1.17
Hospital-based family violence committees	62.6	3.60	1.22
Child investigators	55.6	3.39	1.38
Child protection officers	53.9	3.29	1.37
Police	45.5	3.17	1.37
3. Need professional skills so that I can accurately identify		3.75	1.04
Sexual abuse	67.0	3.88	1.11
Emotional abuse	66.7	3.74	1.14
Neglect	65.2	3.72	1.16
Physical abuse	59.6	3.66	1.17
4. Need knowledge and skills to help me		3.66	0.89
Talk to children in order to examine suspicion	74.4	3.99	1.12
Deal with child's reactions during examination	72.2	3.94	1.21
Deal with parents' reactions to allegations	71.9	4.00	1.10
Work with an interdisciplinary team	70.8	3.91	1.18
Talk with parents	65.6	3.71	1.19
Identify non-accidental injury	62.2	3.71	1.09
Identify inconsistencies in parents' reports on accidents	50.0	3.33	1.25
Discern suspicious physical and behavioral signs	42.0	3.22	1.22
Identify failure to thrive	39.3	3.12	1.20
5. Need knowledge and skills to help monitor and follow up		3.45	1.00
How to testify in court	78.2	4.05	1.20
How to prepare a professional report	67.4	3.73	1.18
What to include in the report	49.4	3.17	1.37
To whom I should report	37.5	2.85	1.44

*Need training to "a large extent" and "to a very large extent"
**Means on a scale 1 = not at all to 5 = to a very large extent

the intensity of the professional involvement with issues of child abuse and neglect. The study strongly indicates that health professionals who are significantly more involved with such cases in their daily work express fewer needs for training. Conversely, those who do not face such cases regularly express a stronger need for training.

It seems, therefore, that although both groups of professionals need training, programs should be tailored to fit their specific needs. That is, health professionals who in their regular daily work are not exposed to many cases of child maltreatment may need a more basic training to ensure that they are aware of signs of abuse and neglect and have rudimentary knowledge in reporting procedures. Professionals who are in positions of greater involvement with such cases should receive a more intensive training, including the acquisition of more sophisticated skills required for assessing and making decisions in complex cases. Such training may include simulations and supervised practice. This recommendation is in line with a growing trend among physicians and other health professionals to specialize in the area of child maltreatment.

With regard to the limitations of the study, the sample used was a convenience sample, which does not represent the overall health professional body in Israel as it over-represents professionals who have a special interest in child maltreatment. Another limitation was that the study was based only on self-report of the health professionals and did not include observations of their actual practice. Nonetheless, this is a step toward a better understanding of the needs of Israeli health professionals in this area.

Future studies should include a representative sample, perhaps stratified on the level of involvement with the field of child abuse. The findings of such a study can serve as a basis for a national training program for health professionals in various practice settings and specialties, such as family physicians, pediatricians and dentists in the community on the one hand and hospital-based physicians and nurses on the other. The efforts to prevent and intervene in cases of child abuse and neglect require the coordinated efforts of professionals from many fields and in a range of settings and roles. Training should be tailored to the specific needs of all relevant professionals in order to achieve a better outcome for maltreated children.

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References

1. Glasser S, Chen W. Survey of a pediatric hospital staff regarding cases of suspected child abuse and neglect. *IMAJ Isr Med Assoc J* 2006; 8: 179-83.
2. Bannon MJ, Carter YH, Barwell F, Hicks C. Perceptions held by general practitioners in England regarding their training needs in child abuse and neglect. *Child Abuse Rev* 1999; 8 (4): 276-83.
3. Shefet D, Dascal-Weichhendler H, Rubin O, et al. Domestic violence: a national simulation-based educational program to improve physicians knowledge, skills and detection rates. *Med Teach* 2007; 29 (5): e133-8.
4. Shor R. Pediatricians in Israel: factors which affect the diagnosis and reporting of maltreated children. *Child Abuse Negl* 1998; 22(2): 143-53.
5. Borres MP, Hägg A. Child abuse study among Swedish physicians and medical students. *Pediatr Int* 2007; 49 (2): 177-82.
6. Leventhal JM. The challenges of recognizing child abuse: seeing is believing. *JAMA* 1999; 281(7): 621-6.
7. Bannon MJ, Carter YH. Pediatricians and child protection: the need for effective education and training. *Arch Dis Child* 2003; 88 (7): 560-2.
8. Van Haeringen AR, Dabbs M, Armstrong KL. The child abuse lottery: will the doctor suspect or report? Physicians' attitudes towards and reporting of suspected child abuse. *Child Abuse Neglect* 1998; 2 (3): 159-69.
9. Lazenbatt A, Freeman R. Recognizing and reporting child physical abuse: a survey of primary health care professionals. *J Adv Nurs* 2006; 56(3): 227-36.
10. Barnard-Thompson K, Leichner P. Psychiatric residents' views on their training and experience regarding issues related to child abuse. *Can J Psychiatry* 1999; 44(8): 769-74.
11. Biehler JL, Apolo J, Burton L. Views of pediatric emergency fellows and fellowship directors concerning training experiences in child abuse and neglect. *Pediatr Emerg Care* 1996; 12(5): 365-9.
12. Giardino AP, Brayden RM, Sugarman JM. Residency training in child sexual abuse evaluation. *Child Abuse Negl* 1998; 22(4): 331-6.
13. Ladson S, Johnson CF, Doty RE. Do physicians recognize sexual abuse? *Am J Dis Child* 1987; 141(4): 411-15.
14. Parra JM, Huston RL, Foulds DM. Resident documentation of diagnostic impression in sexual abuse evaluations. *Clin Pediatr (Phila)* 1997; 36(12): 691-4.
15. Limbos MA, Berkowitz CD. Documentation of child physical abuse: how far have we come? *Pediatrics* 1998; 102(1 Pt 1): 53-8.
16. Wills R, Ritchie M, Wilson M. Improving detection and quality of assessment of child abuse and partner abuse is achievable with a formal organizational change approach. *J Paediatr Child Health* 2008; 44(3): 85-6.
17. Woolf A, Taylor L, Melnicoe L, et al. What residents know about child abuse? Implications of a survey of knowledge and attitudes. *Am J Dis Child* 1988; 142(6): 668-72.
18. King G, Reece R, Bendel R, Patel V. The effects of sociodemographic variables, training, and attitudes on the lifetime reporting practices of mandated reporters. *Child Maltreat* 1988; 3: 276-83.
19. Marshall WN, Locke C Jr. Statewide survey of physician attitudes to controversies about child abuse. *Child Abuse Negl* 1997; 21(2): 171-9.
20. Morris JL, Johnson CF, Clasen M. To report or not to report. Physicians' attitudes toward discipline and child abuse. *Am J Dis Child* 1985; 139(2): 194-7.
21. Zellman GL. Child abuse reporting and failure to report among mandated reporters. *J Interpers Violence* 1990; 5 (1): 3-22.
22. Zellman GL. Report decision-making patterns among mandated child abuse reporters. *Child Abuse Negl* 1990; 14(3): 325-36.
23. Bar-on ME. Teaching residents about child abuse and neglect. *Acad Med* 1998; 73(5): 573-4.
24. Lawrence LL, Brannen SJ. The impact of physician training on child maltreatment reporting: a multi-specialty study. *Mil Med* 2000; 165(8): 607-11.
25. Vulliamy AP, Sullivan R. Reporting child abuse: pediatricians' experiences with the child protection system. *Child Abuse Negl* 2000; 24(11): 1461-70.

"Earth is here so kind, that just tickle her with a hoe and she laughs with a harvest"

Douglas William Jerrold (1803-1857), English dramatist and writer

Validity of Self-Reported Weight and Height among 13–14 Year Old Schoolchildren in Israel

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ABSTRACT: **Background:** Data regarding the validity of self-reported weight and height in adolescents are conflicting.

Objectives: To evaluate the validity of self-reported weight and height among 13–14 year old schoolchildren.

Methods: We conducted a cross-sectional study of 517 schoolchildren aged 13–14 years and compared self-reported and measured weight and height by gender, population group, parental education and crowdedness.

Results: Females under-reported their weight on average by 0.79 ± 5.46 kg ($P = 0.03$), resulting in underestimation of the body mass index with borderline significance (mean difference 0.28 ± 2.26 kg/m², $P = 0.06$). Males over-reported their height on average by 0.75 ± 5.81 cm ($P = 0.03$). Children from less crowded homes (≤ 1 person per room) overestimated their height more than children from more crowded homes, resulting in a significant underestimation of BMI (mean difference between reported BMI and measured values was 0.30 ± 2.36 kg/m², $P = 0.04$). Measured BMI was a significant predictor of the difference between self-reported and measured BMI, adjusted for gender, population group, parents' education, and crowdedness ($\beta = -0.3$, $P < 0.0001$). As a result of this reporting bias, only 54.9% of children with overweight and obesity (BMI ≥ 85th percentile) were classified correctly, while 6.3% of children were wrongly classified as overweight and obese. The largest difference in BMI was observed in obese females (4.40 ± 4.34) followed by overweight females (2.18 ± 1.95) and underweight females (-1.38 ± 1.75). Similar findings were observed for males, where the largest difference was found among obese males (2.83 ± 3.44).

Conclusions: Studies based on self-reported weight and height in adolescents may be biased. Attempts should be made to correct this bias, based on the available data for each population.

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KEY WORDS: body mass index, weight, height, validity, schoolchildren

BMI = body mass index

Self-reported weight and height are common epidemiologic parameters that are used as an alternative to direct measurements both in adults and adolescents. Conflicting results were reported from studies conducted in adolescents. Some found under-reporting of weight and over-reporting of height [1,2], while others found that both height and weight were under-reported [3,4]. The validity of self-reported weight and height in adolesents has not been assessed in Israel. The aim of the present study was to assess the validity of these parameters among 8th grade schoolchildren in Israel.

PATIENTS AND METHODS

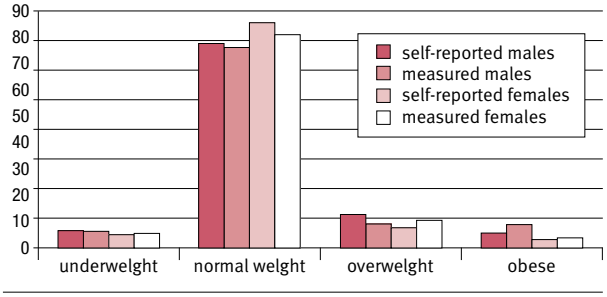
The Israel Center for Disease Control, in collaboration with the Ministry of Education, conducted a national study in 2003 to evaluate the prevalence of asthma in a representative sample of 8th grade schoolchildren in Israel [5]. We collected information on children's height and weight in 11 randomly selected schools. All children were measured and weighed by the school nurses. Body mass index was calculated by dividing weight in kilograms by height (m^2). BMI percentile was determined using a standard growth chart published and developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, Center for Disease Control in 2000 [6]. Self-reported and measured weight, height and calculated BMI were compared by gender, population group, parental education, and crowdedness (defined as the number of persons per room) as an indicator of socioeconomic level. Population groups were "Jews and others" ("others" include non-Arab Christians) and Arabs (Moslems, Christians, Druze and Bedouins). We excluded from the analysis questionnaires where the differences between self-reported and measured height and weight were larger than 4 standard deviations from the mean differences as reported before [7].

RESULTS

Of the 947 schoolchildren measured, 532 had reported their weight and height (56%). Fifteen questionnaires were excluded

since the differences between self-reported and measured height and weight were larger than 4 SD from the mean differences. Analysis of responders and non-responders revealed that parents' education was in general lower in non-responders compared with responders ($P = 0.03$). In addition, compliance of Arab schoolchildren was better than of Jewish schoolchildren ($P < 0.0001$). Females underestimated their weight on average by -0.79 ± 5.46 kg ($P = 0.03$), resulting in underestimation of the BMI (mean difference -0.28 ± 2.26 kg/m², $P = 0.06$). Males overestimated their height on average by 0.75 ± 5.81 cm ($P = 0.03$), and their weight by 0.60 ± 6.91 kg ($P = 0.15$); There were no significant changes in reported versus measured BMI (mean difference 0.04 ± 2.39 kg/m², $P = 0.8$). Significant correlations were observed between the reported and measured height and weight in both genders (Pearson's correlation coefficients ranged between 0.76 and 0.84 for all parameters, in males and females). Children in families where one parent had ≤ 12 years of education and the other > 12 years significantly underestimated their weight, on average, by -0.89 ± 4.57 kg ($P = 0.04$). This resulted in underestimation of BMI by an average of -0.31 ± 1.70 kg/m² ($P = 0.05$). Correlations between self-reported and measured parameters were lower in children of parents with low educational level (≤ 12 years) compared to parents with a higher educational level. Children from less crowded homes (≤ 1 person per room) significantly overestimated their height (mean difference 0.72 ± 5.65 cm, $P = 0.04$), resulting in a significant underestimation of BMI (mean difference -0.30 ± 2.36 kg/m², $P = 0.04$). An overestimation, albeit not statistically significant, of weight was observed in children from more crowded homes (0.45 ± 5.25 kg, $P = 0.22$). Population group differences between self-reported and measured weight and height were not statistically significant. Overestimation of height was on average 0.3 cm among Jews and others and 0.5 cm among Arabs. Correlations between self-reported and measured parameters were higher among Jews and others compared with Arabs. Measured BMI was a significant predictor of the difference between self-reported and measured BMI, adjusted for gender, population group, parents' education, and crowdedness ($\beta = -0.3$, $P < 0.0001$). The largest difference between means of self-reported and measured BMI was observed in obese females (4.40 ± 4.34 kg/m²) followed by overweight females (2.18 ± 1.95 kg/m²) and underweight females (-1.38 ± 1.75 kg/m²). Similar findings were observed for males, where obese males (2.83 ± 3.44 kg/m²) demonstrated the largest difference among males. Significant differences between mean differences were observed between most of the BMI percentile groups in females except between overweight vs. obese and underweight vs. normal weight females ($P < 0.008$ for other groups). In males, significant differences were observed between obese and underweight males and between obese and normal weight males ($P < 0.008$). Based on self-reported weight and height, 7.3% of females were overweight and 2.6% were obese [Figure 1]. Based on measured weight and height 10.3% were overweight and 3.5% were obese. In males, reported overweight was 12.1% and measured overweight was 8.9%, while obesity was 5.3% (self-reported) and 8.9% (measured). These differences were not significantly different. As a result of this reporting bias only 54.9% of children with overweight and obesity (BMI ≥ 85th percentile) were classified correctly, while 6.3% with normal BMI or underweight were classified as overweight/obese. The sensitivity of self-reported weight and height for being overweight/obese (BMI ≥ 85th percentile) was 50% in females and 58% in males. The specificity was 96.5% for females and 91.3% for males, the positive predictive value 69.6% and 59.2% for females and males, respectively, and the negative predictive value 92.3% for females and 90.9% for males.

Figure 1. Self-reported and measured BMI by BMI category in females and males (y axis: %)



DISCUSSION

In the present study BMI based on self-reported weight and height was slightly underestimated in females, but not in males. The degree of bias correlated with the BMI categories. The sensitivity of self-reported weight and height for predicting overweight and/or obesity (BMI ≥ 85th percentile) was low. High correlations were found in the present study between self-reported and measured weight, height and BMI in females and males; however, high correlations do not necessarily indicate valid results, as previously reported [8-10]. Although there were some differences between responders and non-responders in parents' education and population group, these parametes were not associated with the dependent variable and therefore did not bias the results significantly.

In conclusion, self-reported weight and height in adolescents can be misclassified, especially in certain subgroups, resulting in bias esimates of overweight and obesity. This should be considered when analysing data from large self-reported surveys. Some of the results in the present study did not reach statistical significance possibly due to the small sample size. We recommend that this study be repeated in a larger population group in Israel.

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References

1. Brener ND, Mcmanus T, Galuska DA, Lowry R, Wechsler H. Reliability and validity of self-reported height and weight among high school students. *J Adolesc Health* 2003; 32: 281-7.
2. Giacchi M, Mattei R, Rossi S. Correction of the self-reported BMI in a teenage population. *Int J Obes Relat Metab Disord* 1998; 22: 673-7.
3. Strauss RS. Comparison of measured and self-reported weight and height in a cross-sectional sample of young adolescents. *Int J Obes Relat Metab Disord* 1999; 23: 904-8.
4. Himes JH, Faricy A. Validity and reliability of self-reported stature and weight of US adolescents. *Am J Hum Biol* 2001; 13: 255-60.
5. Romano-Zelekha O, Graif Y, Garty BZ, Livne I, Green MS, Shohat T. Trends in the prevalence of asthma symptoms and allergic diseases in Israeli adolescents: results from a national survey 2003 and comparison with 1997. *J Asthma* 2007; 44(5): 365-9.
6. Centre of Disease Control, 2000 growth charts: United States: http://www.cdc.gov/GROWTHCHARTS/.
7. Niedhammer I, Bugel I, Bonenfant S, Goldberg M, Leclerc A. Validity of self-reported weight and height in the French GAZEL cohort. *Int J Obes Relat Metab Disord* 2000; 24: 1111-18.
8. Tokmakidis SP, Christodoulos AD, Mantzouranis NI. Validity of self-reported anthropometric values used to assess body mass index and estimate obesity in Greek school children. *J Adolesc Health* 2007; 40: 305-10.
9. Tsigilis N. Can secondary school students' self-reported measures of height and weight be trusted? An effect size approach. *Eur J Public Health* 2006; 16: 532-5.
10. Elgar FJ, Roberts C, Tudor-Smith C, Moore L. Validity of self-reported height and weight predictors of bias in adolescents. *J Adolesc Health* 2005; 37: 371-5.

Capsule

Elucidating the chromosome 9 association with AS; CARD9 is a candidate gene

Ankylosing spondylitis (AS) is polygenic with contributions from the immunologically relevant genes *HLA-B**27, *ERAP*1 and *IL23R*. A recent genome-wide association screen (GWAS) identified associations ($P = 0.005$) with the non-synonymous single-nucleotide polymorphisms (nsSNPs), rs4077515 and rs3812571, in caspase recruitment domain-containing protein 9 (*CARD*9) and small nuclear RNA-activating complex polypeptide 4 (*SNAPC*4) on chromosome 9q that had previously been linked to AS. Pointon et al. replicated these associations in a study of 730 AS patients compared with 2879 historic disease controls [rs4077515, $P = 0.0004$, odds ratio (OR) 1.2, 95% confidence interval (CI) 1.1–1.4; rs3812571, $P = 0.0003$, OR 1.2, 95% CI 1.1–1.4]. Meta-analysis revealed strong associations of both SNPs with AS, rs4077515, $P = 0.000005$, OR 1.2, 95% CI 1.1–1.3 and rs3812571, $P = 0.000006$, OR 1.2, 95% CI 1.1–1.3. The researchers then typed 1604 AS cases and 1020 controls for 13 tagging SNPs; 6 showed at least nominal association, 5 of which were in *CARD*9. We imputed genotypes for 13 additional SNPs but none was more strongly associated with AS than the tagging SNPs. Finally, interrogation of an mRNA expression database revealed that the SNPs most strongly associated with AS (or in strong linkage disequilibrium) were those most associated with *CARD*9 expression. *CARD*9 is a plausible candidate for AS given its central role in the innate immune response.

Genes Immun 2010; 11: 490

Eitan Israeli

Capsule

Circadian clock regulates blood lipids levels

Circadian regulation of metabolism is emerging as a major homeostatic mechanism. A recent study indicates that a protein that regulates lipid levels in the blood is also under the control of the body clock. Xiaoyue Pan and co-workers examined the role of clock genes – the master regulators of circadian rhythmicity – in the control of a molecule important for keeping the blood levels of triglycerides in check: microsomal triglyceride transfer protein (MTP). MTP acts as chaperone of triglyceride-rich apolipoprotein B lipoproteins; in the absence of MTP, the plasma concentrations of triglycerides decrease and vice versa. The authors used *Clockmt/mt* mice, which express a dominant-negative form of the protein, and found constant hypertriglyceridemia and high levels of MTP. This effect was due to the activation of the MTP promoter and reduced levels of the MTP repressor *Shp*. Indeed, whereas rhythmic variations in *Shp* and MTP levels were inversely correlated, these variations were abrogated in *Clockmt/mt* mice. Furthermore, expression of *Shp* reduced hypertriglyceridemia in *Clockmt/mt* mice.

Cell Metab 2010; 12: 174

Eitan Israeli

"Nobody made a greater mistake than he who did nothing because he could do only a little"

Edmund Burke (1729-1797), Irish statesman, author, orator, political theorist and philosopher

The Effect of Parity and Gravidity on the Outcome of Medical Termination of Pregnancy

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ABSTRACT: **Background:** Previous pregnancies may influence the success of medical termination of pregnancy.

Objectives: To determine the effect of parity and gravidity on the successful termination of pregnancy using mifepristone and misoprostol.

Methods: The medical files of all patients attending a department of obstetrics and gynecology during the years 2006 and 2007 for the purpose of medical termination of pregnancy at ≤ 49 days of gestation were analyzed retrospectively. The medical history, previous pregnancies and deliveries were recorded. Mifepristone was administered orally followed by 400 mg of misoprostol 48 hours later. A second dose of misoprostol was offered 2 weeks later if uterine content thickness was more than 15 mm. Then, after 24 hours, if uterine content thickness was more than 15 mm the uterus was evacuated by dilation and curettage.

Results: Of 403 women, 349 (86.6%) aborted following the basic regime; 207 (51.4%) (group A) were primiparous while 196 (48.6%) (group B) had at least one prior pregnancy. Uterine curettage was performed in 17 patients (8.2%) in group A and in 37 (18.9%) in group B ($P = 0.002$). When patients with a history of a previous abortion were excluded from group B, 32 of 143 (22.4%) required curettage ($P < 0.001$). When patients without a history of previous cesarean section were excluded, 10 of 52 (19.2%) underwent curettage ($P = 0.038$).

Conclusions: Previous pregnancies negatively affect the success of medical termination of pregnancy, especially in women with a previous term pregnancy. This information is important when counseling women about the method of pregnancy termination.

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KEY WORDS: parity, termination of pregnancy, misoprostol, mifepristone

Medical termination of pregnancy using mifepristone and misoprostol or other prostaglandin analogs has gained wide acceptance in recent years. Approximately 92%–97.7% of pregnant women will successfully abort using only mifepristone/misoprostol [1-3]. The failure rate increases with advanced gestational age [1,2] and with a history of previous elective termination of pregnancy [1], previous live birth [2], older age, previous spontaneous abortion, multigravidity, and earlier follow-up visit [4].

The purpose of our study was to test the effect of gravidity and parity on the success of medical termination of pregnancy using mifepristone/misoprostol in pregnancies of 49 days or less.

SUBJECTS AND METHODS

The files of all the women admitted for medical termination of pregnancy during the years 2006 and 2007 were reviewed retrospectively. The medical history, number of previous pregnancies and deliveries, and mode of delivery were recorded. In all cases mifepristone was administered orally followed by 400 mg of misoprostol 48 hours later. All patients were observed for 4 to 6 hours after misoprostol administration and were scheduled to report to our ultrasound clinic 14 days later. At that visit a vaginal ultrasound was performed and uterine content thickness was measured. If the uterine content thickness was less than 15 mm and vaginal bleeding was not observed, the patients were discharged from follow-up. Otherwise, they were offered another administration of misoprostol or uterine curettage. If misoprostol was administered a vaginal ultrasound was performed 24 hours later, and if uterine content thickness was still more than 15 mm a curettage was performed.

STATISTICAL ANALYSIS

Qualitative data were presented as frequencies and percentages; quantitative data were expressed as mean, standard deviation, median and range. The chi-square test or Fisher's exact test, Student's *t*-test and multivariate logistic regression analysis were used as appropriate. The data were analyzed using the statistical software SPSS 11.5 (Chicago, Illinois, USA), and a P value < 0.05 was considered significant.

RESULTS

Over a 2 year period 403 women underwent medical termination of pregnancy in the obstetrics and gynecology department at the Western Galilee Hospital, Nahariya, Israel. The

retrospective analysis of the charts was approved by the local Institutional Review Board (Helsinki Committee). According to the charts 349 women (86.6%) aborted without surgical intervention. For 207 women (51.4%) this was their first pregnancy (group A) while 196 (48.6%) had had at least one previous pregnancy (group B) [Table 1]. The mean age in group A was, as expected, significantly lower than in group B: 21.7 ± 4.1 and 29.5 ± 6.6 years, respectively ($P < 0.001$). Overall, 54 women (13.4%) needed surgical evacuation of the uterus; all had products of conception in the pathology specimen. A statistically significant difference was found between groups A and B in the need for surgical evacuation of the uterus: 17 (8.2%) patients in group A and 37 (18.9%) in group B ($P = 0.002$). Multivariate regression analysis revealed that women who had had more than one pregnancy were more likely than primigravid women to require uterine evacuation ($P = 0.02$, odds ratio 2.25, confidence interval 95% 1.14–4.44). Age was not found to affect the rate of uterine evacuation ($P = 0.361$).

When the data of patients with a previous abortion were excluded from group B, 32 of 143 (22.4%) underwent curettage, and the difference was statistically significant in comparison to group A ($P < 0.001$). When the data of patients without a history of previous cesarean section were excluded, 10 of 52 (19.2%) underwent curettage, and the difference was statistically significant compared to group A ($P = 0.038$). Only 10 patients in group B had had only one previous cesarean section; 4 (40%) of them needed surgical intervention ($P = 0.009$). In order to further evaluate the effect of previous cesarean section on the success rate of medical termination of pregnancy, patients with at least one previous spontaneous delivery but without a previous cesarean (n=80) were compared to patients with at least one previous cesarean section (n=16). We found that among the former, 20 (25%) needed surgical intervention, while in the latter, 5 (31.3%) underwent curettage. This difference was not statistically significant ($P = 0.332$), neither was the age difference between these two subgroups (32.0 ± 4.9 and 33.4 ± 4.9 years respectively).

Only two patients in group B had had one or more spontaneous abortion without previous delivery. Thus, we were unable to test the effect of previous spontaneous abortion on the success of medical termination. However, 52 patients in group B had at least one termination of pregnancy and no deliveries, and 5 of them (9.6%) underwent surgical evacuation of the uterus; this rate was not statistically different from that in group A ($P = 0.78$).

Multivariate logistic regression analysis was used, including previous spontaneous abortion, previous termination of pregnancy, previous cesarean section and spontaneous delivery as the independent variables and the need for surgical intervention as the dependent variable. Previous cesarean section (OR 2.39, 95% CI 1.103–5.16) and previous spontaneous delivery (OR 2.07, 95% CI 1.11–3.86) were the only variables to affect the success of medical termination of pregnancy, with women who had no previous cesarean or spontaneous delivery more likely to have a complete abortion.

Multivariate logistic regression analysis was carried out using forward selection with the need for surgical intervention as the dependent variable, and age, previous pregnancy, previous spontaneous abortion, previous cesarean section, previous spontaneous delivery and previous termination of pregnancy as the independent variables. It revealed that previous pregnancy was the only variable to affect the need for surgical intervention (OR 2.60, 95% CI 1.41–2.48).

Table 1. Pregnancy and delivery history of women with one pregnancy and women with second and additional pregnancies*

	No. of patients (%)	No. of surgical evacuations (%)	*P*
First pregnancy only	207 (51.4%)	17 (8.2%)	
Second and additional pregnancies	196 (48.6%)	37 (18.9%)	0.002
Second and additional pregnancies without previous abortion	143 (35.5%)	32 (22.4%)	< 0.001
Second and additional pregnancies without previous abortion or cesarean	52 (12.9%)	10 (19.2%)	0.038
One previous cesarean section only	10 (2.5%)	4 (40%)	0.009

*All groups and subgroups were compared to the first pregnancy group (See text for more comparisons and for multivariate logistic regression analysis)

DISCUSSION

The use of mifepristone and misoprostol for medical termination has gained wide acceptance by patients and doctors in recent years, and as medical termination of pregnancy increases it is becoming more important to be able to characterize patients at high risk for failure.

Our study clearly demonstrates that after administration of mifepristone/misoprostol for medical termination of pregnancy, women with any previous pregnancy, especially term pregnancies – whether ended by cesarean section or spontaneous delivery – are more likely to require surgical intervention compared to primigravid women. This result is in agreement with several previously published reports [2,4,5,6], although some reports did not find any correlation between parity and the need for surgical intervention [7,8].

The overall need for surgical uterine evacuation was relatively high (13.4%) compared to other studies [1-3]; this may be attributed to the fact that we administered misoprostol orally and not vaginally. The difference between these two

OR = odds ratio
CI = confidence interval

routes of administration was reported by El-Refaey et al. [9], who found that 87% of patients receiving misoprostol orally aborted, while vaginal administration of misoprostol resulted in a 95% abortion rate.

Ashok and colleagues [2] found that women with a previous abortion were more likely to have a failed medical termination, while in the present study previous abortions, whether spontaneous or induced, did not affect the rate of successful medical termination. Furthermore, others reported that older age was also associated with a higher failure rate of medical termination of pregnancy [4], while in our study, as in that of Ashok et al. [2], age had no effect on the success rate. However, as long as there is no biologic mechanism to explain the effect of previous pregnancy and age on the success rate of medical termination, these observations need further investigation.

Attempting to predict the success rate of medical termination by measuring beta-human chorionic gonadotropin levels and endometrial thickness a few days after administration of misoprostol, some researchers [4,10] found that β-HCG levels and endometrial thickness were higher among the failures. However, the predictive values of these tests were low [10] and could not be used clinically as diagnostic tests in predicting late failure after medical abortion.

A weakness of this study was that we did not contact the patients to check if they underwent surgical uterine evacuation at another medical center. However, in the area we serve (the Western Galilee), our department is the only Obstetrics and Gynecology service; hence only on rare occasions would women travel elsewhere to undergo uterine evacuation. In addition, patients undergoing this procedure pay in advance, and the fee includes a surgical uterine evacuation if needed, as well as the management of any complications of the procedure. Furthermore, the hospital's Institutional Review Board did not permit phoning patients because of privacy and confidentiality considerations related to previous pregnancy.

From this and other studies it is clear that before consulting patients about the route of termination of pregnancy, data on previous pregnancies are a determining factor in failure of medical termination and this fact should be considered and disclosed to patients before a decision is made on the route of abortion. The impact of other variables, such as age, previous abortions and previous termination of pregnancy, need to be investigated in further studies.

B-HCG = beta-human chorionic gonadotropin

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References

1. Spitz IM, Bardin W, Benton L, Robbins A. Early pregnancy termination with mifepristone and misoprostol in the United States. *N Engl J Med* 1998; 338: 1241-7.
2. Ashok PW, Templeton A, Wagaarachchi Pt, Flett GMM. Factors affecting the outcome of early medical abortion: a review of 4132 consecutive cases. *Br J Obstet Gynaecol* 2002; 109: 1281-9.
3. Ulman A, Silverstre L, Chemama L, et al. Medical termination of early pregnancy with mifepristone (RU 486) followed by a prostaglandin analogue; study in 16369 women. *Acta Obstet Gynecol Scand* 1992; 71: 278-83.
4. Haimov-Kochman R, Arbel R, Sciaky-Tamir Y, Berzezinski A, Laufer N, Yagel S. Risk factors for unsuccessful medical abortion with mifepristone and misoprostol. *Acta Obstet Gynecol Scand* 2007; 86: 462-6.
5. Child TJ, Thomas J, Rees M, MacKenzie IZ. A comparative study of surgical and medical procedures: 932 pregnancy terminations up to 63 days gestation. *Hum Reprod* 2001; 16: 67-71.
6. Bartley J, Tong S, Everington D, Baird DT. Parity is a major determinant of success rate in medical abortion: a retrospective analysis of 3161 consecutive cases of early medical abortion treated with reduced doses of mifepristone and vaginal gemeprost. *Contraception* 2000; 62: 297-303.
7. The efficacy and tolerance of mifepristone and prostaglandin in termination of pregnancy of less than 63 days gestation; UK multicentre study – final results. *Contraception* 1997; 55: 1-5.
8. Hill NCW, Ferguson J, MacKenzie IZ. The efficacy of oral mifepristone (RU 38,486) with a prostaglandin E1 analog vaginal pessary for termination of early pregnancy: complications and patient acceptability. *Am J Obstet Gynecol* 1990; 162: 414-17.
9. El-Refaey H, Rajasekar D, Abdalla M, Calder L, Templeton A. Induction of abortion with mifepristone (RU486) and oral or vaginal misoprostol. *N Engl J Med* 1995; 332: 983-7.
10. Rorbye C, Norgaard M, Nilas L. Prediction of late failure after medical abortion from serial B-HCG measurements and ultrasonography. *Hum Reprod* 2004; 19: 85-9.

Capsule

Artificial corneal transplants improve sight

Artificial corneal transplants improved the sight of more than 50% of patients with vision loss in a small 2 year clinical trial. Corneal disease and damage are two of the main causes of vision loss and blindness, and affect millions worldwide. Due to the huge shortage of human donor corneas, Griffith at Linköping University in Sweden and her colleagues created biosynthetic corneas from human collagen. Unlike plastic corneas, the biosynthetic ones mimic the cornea's protein scaffolding, triggering regeneration of the patient's own corneal cells and nerve growth in the eye. In the study of 10 patients who received the implant, visual acuity improved in 6, remained the same in 2 and decreased in 2. All 10 could further correct their vision with contact lenses.

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Eitan Israeli

Achilles Tendon Rupture and our Experience with the Achillon Device

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ABSTRACT: **Background:** Open repair of the Achilles tendon is still the gold standard for treating rupture. This technique has the disadvantages of a long and problematic operative scar and thickly scarred Achilles tendon. To improve the surgical outcome minimally invasive techniques have been developed.

Objectives: To analyze our results of Achilles tendon repair using the Achillon® device and compare them with published studies.

Methods: We performed surgical repair of the Achilles tendon in 28 patients during a 4 year period (2004–2008): 14 patients were treated with the Achillon device, 12 with the open suture technique and 2 with the percutaneous method. Fourteen patients were available for follow-up: 9 patients with the Achillon device, 3 patients with open suturing and 2 patients with the percutaneous technique. Follow-up ranged from 1 to 4 years.

Results: The average score of the AOFAS Ankle-Hindfoot Scale for the group treated with the Achillon device was 95.6 points (range 84–100) and for the group treated with the open method, 90 points (range 84–98). The length of the scar in patients operated with a minimally invasive technique was 3.81 cm (range 1–6 cm) as compared to 9.16 cm (range 8–10.5 cm) with the open suture.

Conclusions: This is the first review on this procedure in Israel. Excellent functional results were achieved with this technique. Our outcomes were similar to those of two other studies.

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KEY WORDS: Achilles, Achillon™, percutaneous method, minimally invasive technique

One of the central heroes of Homer's *Iliad* [1], a classic poem about the Trojan War written in ancient Greek, is Achilles. Epitomizing the perfect warrior, who is fearless, sincere, honest and willing to die in the service of his friends and allies, Achilles was the son of a goddess and a human. His mother, Thetis, plunged him into the river Stix to make him immortal. Unfortunately, she held him by the heel, which did not get immersed and thus remained the only vulnerable part of his body. This set of circumstances will lead to his death in a battle outside the walls of the city of Troy due to being struck in the heel by a poisoned arrow. Since then, "Achilles' heel" has come to mean an individual's principal weakness.

The Achilles tendon, formed from tendinous contributions of the gastrocnemius and soleus muscles, is the largest and strongest tendon in the human body. The tendons converge approximately 15 cm proximal to the insertion at the posterior calcaneus [2]. The Achilles tendon is stiff but resilient, possesses a high tensile strength, and has the ability to stretch up to 4% before damage occurs. When stretch exceeds 8%, macroscopic rupture occurs [3,4]. The midsection of the Achilles tendon is markedly more hypovascular than the rest of the tendon, and the risk of rupture and surgical complications is therefore highest at its midsection. Individuals with particularly poor blood supply of the midsection may also be at increased risk of tendon rupture [5].

Although ruptures of the Achilles tendon are relatively common, the incidence in the general population is difficult to determine but has probably risen in the past decade due to the increased popularity of recreational sports. Leppilahit et al. [6] studied the incidence of ruptures of the Achilles tendon in the city of Oulu, Finland, over the 16 year period 1979–1994, during which 110 ruptures occurred. The incidence increased from 2 ruptures/10^5 inhabitants in 1979–1986 to 12 in 1987–1994, with a mean of 7. The peak annual incidence of 18 was recorded in 1994. The incidence was highest in the age group 30–39 years. Male dominance was 5.5:1, and 81% of the ruptures were related to sports, with 88% occurring in ball games.

For many years conservative treatment was an option for Achilles tear. A known story from the Second World War is a good example of the rupture healing without suturing. British pilots who were captured by the German army made several attempts to escape; the German guards decided to cut their Achilles tendon to prevent their escaping but after a few weeks the tendons healed and the pilots succeeded to flee. Nevertheless, due to the paucity of data on the effectiveness of conservative treatment a surgery department in the Netherlands [7] undertook a randomized prospective study a few years ago to compare operative versus conservative treatment of Achilles tendon. A meta-analysis by Khan et al. [8] comparing operative versus conservative treatment in acute rupture of Achilles tendon concluded that open opera-

tive treatment of acute Achilles tendon ruptures significantly reduces the risk of re-rupture compared with non-operative treatment. On the other hand, surgical treatment is associated with a significantly higher risk of complications. Advanced surgical methods have evolved, and surgery is now the preferred modality for treating ruptured Achilles tendons [9]. The optimal surgical method for ruptured Achilles tendon is still under debate and various techniques are recommended, but due to the lack of prospective randomized trials there is insufficient evidence to determine the best option.

PATIENTS AND METHODS

The use of the Achillon® device (Integra™ Newdeal, France) is recommended in acute ruptures (< 10 days from occurrence of closed injury and < 6 hours from open injury without skin defect), where the tear is in the middle of the tendinous portion of the tendon (2–8 cm proximal to the calcaneal tuberosity). Contraindications to Achillon usage include previous surgery, history of steroid injection, injury < 2 cm and > 8 cm from the calcaneal tuberosity, an uncooperative or pediatric patient, and open rupture > 6 hours or open ruptures with skin defect. The decision as to what method should be used during surgery was based on two key criteria. The first criterion was the location of the tear. This location was initially diagnosed based on a positive Thompson test and palpation of the tendon, further substantiated by ultrasound [10]. All patients underwent X-ray to exclude avulsion fracture and gross calcification. The second criterion was the surgeon's familiarity with the Achillon device.

The Achillon system, as described by Assal [11], comprises a main guiding instrument consisting of a pair of internal branches connected to a pair of external branches; each branch has holes at the same level allowing easy and accurate passage of the sutures through all four branches. Ideally, sutures should be placed as far from the ruptured area as possible to ensure good fixation within the undamaged tendon.

In our department we prefer to operate within 48 hours from a patient's admission. After selection of the operation procedure, the correct affected side is confirmed. A second-generation medication, cephalosporin, is administered prophylactically. Through a medial 4 cm longitudinal incision the tendon is revealed after subcutaneous dissection, taking care not to injure the sural nerve. The tendon stumps are identified and careful debridement is made on both ends. The Achillon device is then introduced in the proximal part of the paratenon, and three sutures are placed percutaneously. The Achillon device is then placed under the paratenon of the distal stump until it reaches the calcaneus, and another three sutures are passed. The foot is placed in an equinus position, and the sutures are tightened. Because most of the patients were young and mobile, usually using crutches soon after surgery, the department policy was not to administer prophylactic anticoagulants post-surgery. Although very good results with early range of motion after surgery with the Achillon device have been published, we use a more conservative rehabilitation protocol. Immediately after surgery the foot is placed in a cast or below-knee orthotic in plantar flexion of 30 degrees for 2 weeks. After this interval the position is changed to neutral, and no weight bearing is allowed for another 4 weeks. During this period the patient begins physiotherapy for movements ranging between plantar and neutral positions. After 6 weeks, partial weight bearing is allowed.

During a 4 year period (2004–2008), 28 patients underwent surgical repair of Achilles tendon in our department: 14 were treated by the Achillon device, 12 by open suturing and 2 patients underwent percutaneous repair. The age in the Achillon group ranged from 30 to 62 years (mean 45), and in the open group from 16 to 62 (mean 44). Fourteen patients were available for follow-up: 9 treated by the Achillon device, 3 by open repair and 2 percutaneously. The follow-up ranged from 1 to 4 years, median 2.5 years

Most of the patients ruptured their Achilles tendon during recreational sport, especially football, and one patient tore the Achilles tendon while dancing. It is worth mentioning that none of the patients were regular sports enthusiasts and some of them played only occasionally. One of the patients was not available for a physical examination and was interviewed by phone.

The AOFAS Ankle-Hindfoot Scale was used to evaluate the patients, and two more criteria, as suggested by Kitaoka and collaborators [12], were added: neurovascular status of the foot and strength of plantar flexion. There was a clear male predominance in this series, 12 of the 14 patients. Six of the patients were employed in jobs requiring physical labor. None of the patients felt pain or had problems in the affected side before the injury.

RESULTS

The average score of the AOFAS Ankle-Hindfoot Scale in the group with the Achillon device was 95.6 (range 84–100). Even though the open group was small, we looked at the score, which averaged 90 points (range 84–98). Our outcome with the Achillon was similar to the 96 points recorded in the study by Assal et al. [11] and 96.8 in the research of Aktas et al. [22]. The length of the scar in patients operated with a minimally invasive technique (Achillon device) was 3.81 cm (range 1–6 cm) compared to 9.16 cm (range 8–10.5) with open suturing.

All patients who underwent surgery with the Achillon device were neurovascular intact, and none had re-ruptured. Two patients suffered from hypercoagulabillity complications: one (age 44 years) had pulmonary embolism and the other (age 49) had deep vein thrombosis. Neither had previously known cardiovascular risk factors. Those complications

occurred in the third week postoperatively in both patients. All the patients have returned to their pre-injury level of activity; none had played sports professionally.

DISCUSSION

Various surgical techniques have been described for treating ruptured Achilles tendon. A review of the literature yielded the following procedures:

- The Open Method usually uses the Krackow suture [13] named after its inventor, Kenneth A. Krackow and published in 1986. Since then most open Achilles tendon repairs were performed this way. Other suture methods were suggested: "Gift Box" sutures by Labib et al. [14] were tested in a biomechanical study. Achilles tendons repaired with this Gift Box technique were more than twice as strong as those repaired with the traditional Krackow technique.
- Repairing Achilles tendon rupture with fibrin glue has been used since the 1980s. A recent study by Hohendorff and team [15] showed good results, with no difference for long-term evaluation if augmentation with the plantaris longus tendon was performed.
- In 1977, Ma and Griffith [16] were the first to describe a percutaneous technique for the repair of acute Achilles tendon rupture, and this technique became popular with modification as described by Blankstein and co-workers [17] using real-time sonography. In 1992, Delponte et al. [18] presented a new percutaneous technique with special material: a Dacron yarn with a 5 mm wide hook set on a 12 long flexible needle.
- Percutaneous repair of the Achilles tendon ruptures reportedly reduces the risk of re-rupture compared to non-operative treatment and reduces the risk of wound infection compared to open surgery [19]. However, using this surgical method carries a greater risk of injury to the sural nerve [20].
- A retrospective study by Maffulli and colleagues [21] suggested that percutaneous repair of the Achilles tendon is a suitable option for patients older than 65, producing similar outcomes when compared to percutaneous repair in younger patients.
- Recently, various apparatuses for minimal invasive repair of the Achilles tendon are being proposed.
- In 2002 the minimally invasive method using the Achillon device was developed by Assal et al. [12]. This method is a combination of the open and percutaneous method and essentially improved the suturing technique without incurring the disadvantages of those two methods. The open method uses a long longitudinal surgical approach, averaging 10 cm, which has been shown to encroach upon an area of poor vascularity, resulting in a higher rate of superficial and deep wound infection and delayed wound healing. The percutaneous repair bears a high risk of sural nerve entrapment and rerupture. The Achillon device has the advantage of direct visualization of the repair site and a small incision, averaging about 4 cm. A prospective study by Aktas el al. [22] compared the clinical and functional results of patients who underwent open repair and those who underwent repair with the Achillon device. Theirs was the first prospective study since introduction of the minimally invasive method. There was no significant difference between the functional results of the two groups measured by the AOFAS score and no incidence of re-rupture in both groups. The complications encountered were mostly with the open method using Krakow sutures: one case of deep and three of superficial infection, one case of ankle stiffness, one case of deep vein thrombosis, and one large hematoma. In the Achillon group there was one minor complication (insertional tendinopathy). A biomechanical study on cadaveric specimens by Huffard et al. in 2008 [23] demonstrated that the Achillon repair is stronger then the Krackow repair using identical sutures.

Rupture of Achilles tendon is not a rare injury and many treatment options have been advocated. The relatively new minimally invasive surgical technique using the Achillon device has the advantage of the open method, which allows viewing of the adaptation of the tendon and suturing the paratendon envelope. Furthermore, this method does not have the disadvantage and complication of a large scar in a problematic hypovascular area and the associated higher infection rate, or the risk of causing damage to the sural nerve with the other percutaneous method. We began using this method in 2004 and have experienced very positive results. These outcomes and follow-up indicate that this is a safe method and it has an easy learning curve.

Since most of the patients suffering Achilles tear are young and active, post-surgery prophylactic anticoagulants were not administered as a rule. However on follow-up, two patients, 44 and 49 years old without cardiovascular risks, were found to have thromboembolic complications.

Although the results of our study were not statistically significant overall, we compared our Achillon device group to similar groups in the literature. In view of the positive outcome, we believe that a larger study on this issue is needed and, furthermore, suggest that prophylactic anticoagulation treatment be considered for those patients.

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References

1. The Iliad by Homer Greek Mythology, Trojan War. Translated by Samuel Butler, edited by Louise Ropes Loomis. New York: Walter J. Black, Inc. , 1944; xxxix.
2. Jacobs BA, Lin DY, Schwartz E. Achilles tendon ruptures. *Emedicine* 2009.
3. Maffulli N. Rupture of the Achilles tendon. *J Bone Joint Surg Am* 1999; 81: 1019-36.
4. Moore KL. Clinically Oriented Anatomy. 3rd edn. Philadelphia: Lippincott Williams &Wilkins, 1999: 449-51.
5. Chem TM, Rozen WM, Pan WR, Ashton MW, Richardson MD, Taylor GI. The arterial anatomy of the Achilles tendon: anatomical study and clinical implications. *Clin Anat* 2009; 22: 377-85.
6. Leppilahit J, Puranen J, Orava S. Incidence of Achilles tendon rupture. *Acta Orthop Scand* 1996; 67: 277-9.
7. Mertz R, Kerkhoffs GM, Verleisdonk EJ, van der Heijden GJ. Acute Achilles tendon rupture: minimally invasive surgery versus non operative treatment, with immediate full weight bearing. Design of a randomized controlled trail. *BMC Musculoskelet Discord* 2007; 8: 108.
8. Kahn RJ, Fick D, Koegh A, Crawford J, Brammar T, Parker M. Treatment of acute Achilles tendon ruptures. A meta-analysis of randomized, controlled trials. *J Bone Joint Surg Am* 2005; 87: 2202-10.
9. Cetti R, Christensen SE, Ejsted R, Jensen NM, Jorgensen U. Operative versus nonoperative treatment of Achilles tendon rupture. A prospective randomized study and review of the literature. *Am J Sports Med* 1993; 21: 791-9.
10. Blankstein A, Cohen I, Diamant L, et al. Achilles tendon pain and related pathologies: diagnosis by ultrasonography. *IMAJ Isr Med Assoc J* 2001; 3: 575-8.
11. Assal M, Jung M, Stern R, Rippstein P, Delmi M, Hoffmeyer P. Limited open repair of Achilles tendon ruptures. A technique with a new instrument and findings of a prospective multicenter study. *J Bone Joint Surg Am* 2002; 84-A: 161-70.
12. Kitaoka HB, Alexander IJ, Adelaar RS, Nunley JA, Myerson MS, Sanders M. Clinical rating systems for the ankle-hindfoot, midfoot, hallux, and lesser toes. *Foot Ankle Int* 1994; 15: 349-53.
13. Krackow KA, Thomas SC, Jones LC. A new stitch for ligament-tendon fixation. Brief note. *J Bone Joint Surg Am* 1986; 68: 764-6.
14. Labib SA, Rolf R, Dacus R, Hutton WC. The "Giftbox" repair of the Achilles tendon: a modification of the Krackow technique. *Foot Ankle Int* 2009; 30: 410-14.
15. Hohendorff B, Siepen W, Staub L. Treatment of acute Achilles tendon rupture: fibrin glue versus fibrin glue augmented with the plantaris longus tendon. *J Foot Ankle Surg* 2009; 48: 439-46.
16. Ma GW, Griffith TG. Percutaneous repair of acute closed ruptured Achilles tendon: a new technique. *Clin Orthop Relat Res* 1977; 128: 247-55.
17. Delponte P, Potier L, de Poulpiqet P, Buisson P. Treatment of subcutaneous rupture of the Achilles tendon by percutaneous tenorraphy. *Rev Chir Orthop Reparatrice Appar Mot* 1992; 78: 404-7
18. Blankstein A, Israeli A, Dudkiewicz I, Chechick A, Ganeal A. Percutaneous Achilles tendon repair combined with real-time sonography. *IMAJ Isr Med Assoc J* 2007; 9: 83-5.
19. Cretnik A, Kosanović M, Smrkolj V. Percutaneous suturing of the ruptured Achilles tendon under local anesthesia. *J Foot Ankle Surg* 2004; 43: 72-81.
20. Maes R, Copin G, Averous C. Is percutaneous repair of the Achilles tendon a safe technique? A study of 124 cases. *Acta Orthop Belg* 2006; 72: 179-83.
21. Maffulli N, Longo UG, Ronga M, Khanna A, Denaro V. Favorable outcome of percutaneous repair of Achilles tendon ruptures in the elderly. *Clin Orthop Relat Res* 2010; 468: 1039-46.
22. Aktas S, Kocaoglu B. Open versus minimal invasive repair with Achillon device. *Foot Ankle Int* 2009; 30: 391-7.
23. Huffard B, O'Loughlin PF, Wright T, Deland J, Kennedy JG. Achilles tendon repair: Achillon system vs. Krackow suture: an anatomic in vitro biomechanical study. *Clin Biomech* 2008; 23: 1158-64

Capsule

Enhancement of proteasome activity by a small-molecule inhibitor of USP14

Proteasomes, the primary mediators of ubiquitin–protein conjugate degradation, are regulated through complex and poorly understood mechanisms. Lee et al. show that USP14, a proteasome-associated deubiquitinating enzyme, can inhibit the degradation of ubiquitin-protein conjugates both in vitro and in cells. A catalytically inactive variant of USP14 has reduced inhibitory activity, indicating that inhibition is mediated by trimming of the ubiquitin chain on the substrate. A high throughput screen identified a selective small molecule inhibitor of the deubiquitinating activity of human USP14. Treatment of cultured cells with this compound enhanced degradation of several proteasome substrates that have been implicated in neurodegenerative disease. USP14 inhibition accelerated the degradation of oxidized proteins and enhanced resistance to oxidative stress. Enhancement of proteasome activity through inhibition of USP14 may offer a strategy to reduce the levels of aberrant proteins in cells under proteotoxic stress.

Nature 2010; 467: 179

Eitan Israeli

Capsule

Predictions in lymphoma – a new biomarker with potential clinical applications

Irish and co-authors used single-cell profiling in samples of human follicular lymphoma and found a subset of lymphoma cells with defective B cell antigen receptor (BCR) signaling. The more of these cells in each tumor, the shorter the overall subject survival. Moreover, these lymphoma cells increased in number as tumors relapsed after chemotherapy. Interestingly, BCR signaling could be reactivated in the defective cells, indicating that BCR signaling was not altogether absent but somehow suppressed. Mechanistically, tumors with high counts of defective BCR cells had less interleukin-7 signaling in infiltrating T cells, but additional work will be required to understand how these cells emerge and what their downstream effects are.

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Eitan Israeli

Short and Long-Term Outcome of Pregnant Women with Preexisting Dilated Cardiomypathy: an NTproBNP and Echocardiography-Guided Study

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ABSTRACT: **Background:** Little is known of the outcome of pregnant patients with previously diagnosed dilated cardiomyopathy. These patients are usually firmly advised against continuation of the pregnancy.

Objectives: To examine the usefulness of serial echocardiographic follow-up and plasma N-terminal pro-B type natriuretic peptide levels in the management of pregnant women with preexisting DCM.

Methods: We prospectively enrolled pregnant women with DCM either known or diagnosed in the first trimester. Clinical examination and serial echocardiography studies were performed at baseline, at 30 weeks gestation, peripartum, and 3 and 18 months postpartum. Blinded NTproBNP levels were obtained at 30 weeks, at delivery and 3 months postpartum.

Results: Between June 2005 and October 2006 we enrolled seven women who fulfilled the study criteria. Delivery and postpartum were complicated in 3 patients (42%): 2 with acute heart failure, which resolved conservatively, and 1 with major pulmonary embolism. The left ventricular ejection fraction was stable throughout the pregnancy (35% ± 2.8 at baseline, 33% ± 2.9 at 30 weeks) and postpartum (35% ± 2.8 at 1 day, 34% ± 3.1 at 90 days). Similar stable behavior was observed regarding left ventricular dimensions: LV end-systolic diameters 43.3 ± 2.7 mm and LV end-diastolic diameters 57.3 ± 3.3 mm at baseline compared with 44.1 ± 3.1 mm and 58.7 ± 3.1 mm postpartum, respectively. The NTproBNP levels rose significantly peripartum in all three patients with complications.

Conclusions: Serial NTproBNP levels, as compared to echocardiography, may be a better clinical tool in monitoring and management of pregnant women with preexisting DCM. An early rise in NTproBNP level appears to predict the occurrence of adverse events.

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KEY WORDS: pregnancy, preexisting dilated cardiomyopthy, N-terminal pro-B type natriuretic peptide (NTproBNP), echocardiography

Pregnancy in women with known (preexisting) dilated cardiomyopthy has been rarely described, and the clinical management and prenatal care of this patient population are unclear. While some studies reported a good pregnancy outcome and stable cardiac function in patients with DCM, others noted poor clinical outcome including maternal death or heart transplantation [1,2]. The physiologic cardiovascular adaptations during pregnancy are well characterized [3]. The observed increase in cardiac output is primarily due to increases in both heart rate and stroke volume, as demonstrated in several echocardiographic studies [4,5]. B-type natriuretic peptide is an emergent and relatively new clinical tool with diagnostic [6] and prognostic value [7]. In healthy pregnant women BNP levels are in the normal range throughout gestation but may increase to pathologic levels during alterations in hemodynamic homeostasis, such as preeclampsia [8].

We conducted a prospective observational study to examine the usefulness of blinded serial BNP levels and echocardiographic studies as non-invasive tools in the monitoring and management of pregnant women with preexisting or newly developed DCM.

PATIENTS AND METHODS

STUDY DESIGN AND PATIENT POPULATION

The study protocol was approved by the Assaf Harofeh Medical Center Institutional Review Board. We prospectively enrolled all pregnant women with known DCM or those diagnosed in the first trimester. DCM was defined as depressed left ventricular ejection fraction ≤ 45% as determined by transthoracic echocardiography. All women fulfilling this inclusion criterion underwent a comprehensive evaluation by obstetricians and cardiologists, and the possible deleterious effects of pregnancy and/or delivery on their cardiac performance were discussed. Women who elected

DCM = dilated cardiomyopathy
NTproBNP = N-terminal pro-B type natriuretic peptide
LV = left ventricular

to continue their pregnancy and agreed to participate in a prospective follow-up study were enrolled and followed in our high risk pregnancy unit and the cardiology department. Written informed consent was obtained from all patients.

Demographic and clinical characteristics were obtained using questionnaires. Clinical follow-up continued in the outpatient cardiac clinic 3, 6, 12 and 18 months after delivery. Echocardiographic studies were performed at enrollment (baseline), at 30 weeks gestation, immediately after delivery and 3, 6 and 18 months postpartum. All studies were conducted with the VIVID 3, GE Health Care system using a 3.25 MHz transducer. The left ventricle was divided into 16 segments and left ventricular dimensions were measured according to the recommendations of the American Society of Echocardiography, based on a 16 segment model. Ejection fraction was determinated by the modified method of Quinones et al. [9].

NTProBNP LEVELS

Peripheral venous blood samples were obtained at 30 weeks gestation and during the first stage of delivery. The samples were centrifuged and the serum was stored at -70ºC. The NTproBNP analysis was performed on serum using a standard core laboratory assay (Roche Diagnostic, Basel, Switzerland). The medical management team was blinded to the BNP results.

STATISTICAL ANALYSIS

Values are expressed as the median and interquartile range.

RESULTS

Between June 2005 and October 2006 we enrolled seven women fulfilling the study criteria. The mean age was 33.5 ± 3.3 years; six women were Caucasian and one was Ethiopian. DCM had been diagnosed in five patients before the current pregnancy and in two during the first trimester in the course of workup for effort dyspnea. New York Heart Association functional class before the pregnancy was good in all women: NYHA I-II. Medical history revealed hypothyroidism in four patients (57%), pregestational diabetes type 2 in one, and chronic hypertension in another [Table 1]. Four patients (57%) were primagravidas; two of them conceived following infertility treatments. Six patients had singleton gestation and one patient had a twin gestation.

All the women underwent fetal echocardiography during pregnancy, which was normal. None had pregnancy-induced hypertension or any deterioration in their functional class according to the NYHA during the pregnancy. During delivery all women were given epidural analgesia without any complication; four had vaginal delivery and three had cesarean section. All neonates were born at term, had normal Apgar score and pH level, and their weight was appropriate for their gestationl age.

Table 1. Characteristics of patients with preexisting dilated cardiomyopthy

Age (yrs)	33.5 ± 3.3
Ethnicity	
Caucasian	6
Black	1
Risk factors	
Hypertension	1
Diabetes mellitus	1
Gestation-induced	
Hypertension	0
Diabetes mellitus	0
Hypothyroidism	4
NYHA Functional class	
I	4
II	3
Prior heart failure acute decompensation	2
Medication	
None	3
Beta-blocker	2
ACE/ARB	4
Diuretic	1

ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker

The delivery and postpartum were complicated in three patients (42%): two (patients 2 and 5) developed acute heart failure several hours after the delivery and were transferred to the intensive cardiac care unit. These cardiac events resolved following conservative medical therapy. One patient (# 3) developed massive acute pulmonary embolism 3 days after delivery. The patient was treated with tissue plasminogen activator acutely and an inferior vena cava filter was inserted due to recurrent pulmonary embolism despite adequate anticoagulation therapy.

Six patients, including the two with acute heart failure, returned rapidly to the baseline NYHA functional class. The woman with pulmonary embolism became asymptomatic 3 months after delivery.

Left ventricular ejection fraction was stable throughout gestation (35 ± 2.8% at enrollment, 33 ± 2.9% at 30 weeks) and in the early and late postpartum periods (35 ± 2.8% on first postpartum day, 34 ± 3.1% at 90 days, and 33 ± 2.7% at 18 months) [Figure 1]. Similar stable findings were observed in left ventricular dimensions: LVESD 43.3 ± 2.7 mm and LVEDD 57.3 ± 3.3 mm at baseline and 30 weeks gestation, respectively. At 3 and 18 months after delivery echocardiographic measurements did not change significantly: 44.1 ± 3.1 mm, 58.7 ± 3.1 mm and 57.8 ± 2.9 mm.

Figure 2 represents the NTproBNP levels during the study. The NTproBNP levels were above a cutoff of 300 pg/ml before

NYHA = New York Heart Association

LVESD = left ventricular end-systolic diameter
LVEDD = left ventricular end-diastolic diameter

delivery in all patients with complications. These patients presented a "step up" in their 30 week pregnancy NTproBNP levels. In the remaining, event-free four patients, NTproBNP levels remained stable throughout gestation, before delivery and in the immediate postpartum period. One uncomplicated delivery (patient 7, twins pregnancy) showed values above 300 pg/ml, without a significant elevation between samples.

DISCUSSION

Our prospective study found relative safety and good pregnancy and neonatal outcome in this high risk population due to close obstetric and cardiology supervision. The present study demonstrates the potential additional clinical value of serial NTproBNP levels to predict cardiovascular complications in the management of these high risk pregnant women. Serial echocardiography failed to predict adverse outcome in this small study group.

This is no doubt a high risk population. The theoretic estimated risk in this patient group according to the Canadian risk index [10] is > 27% (one or more predictors). Fortunately, this is an infrequent clinical situation, since most women with DCM avoid pregnancy, and some will choose pregnancy termination to avoid the reportedly adverse outcomes. In our relatively large series of women we observed the occurrence of major cardiovascular events in 3 patients (42%). There was, however, no fatal event in the short and long-term follow-up, an observation in concordance with the low mortality rate among patients with peripartum cardiomyopathy [11-13].

We propose the use of a new terminology to more precisely characterize patients with heart failure associated with pregnancy:

- Group 1 – preexisting/newly developed DCM according to our inclusion criteria: either known significant systolic dysfunction beforehand or diagnosed in the initial phase of the pregnancy
- Group 2 – gestational DCM: heart failure developing toward the end of pregnancy or several months after delivery (peripartum cardiomyopathy)
- Group 3 – acute heart failure superimposed on preexisting DCM.

This new classification concurs with the presence of hypertension during the pregnancy: chronic hypertension, gestational hypertension, and preeclampsia/eclampsia superimposed on chronic hypertension.

Serial echocardiography in these women showed no significant differences between studies. It is possible that dilated dysfunctional hearts may have responded differently to normal hearts [3-5].

The cutoff of "normal" NTproBNP values depends on the clinical scenario. Based on Resnik et al. [8] the normal value

Figure 1. LVEF during pregnancy and postpartum

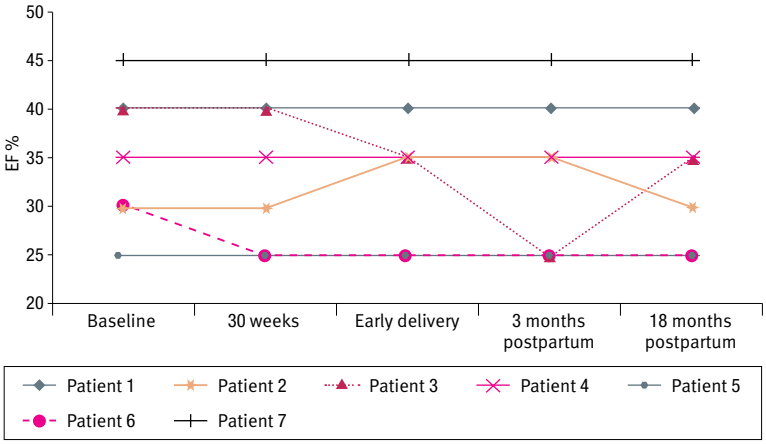
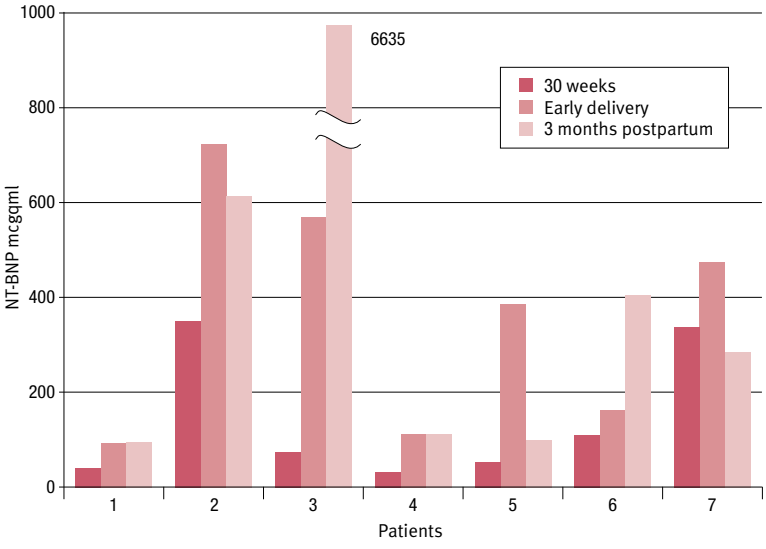


Figure 2. NT-BNP levels during pregnancy and postpartum



of BNP in healthy pregnant wemen (without cardiomyopathy or any other disease) is less than 100 pg/ml. In this sublinical disease – previously asymptomatic women with known depressed systolic function – 300 pg/ml is a reasonable value [14]. Most importantly, the significant increase ("step up") in the peripartum period, compared with the patient's baseline, appears to be a significant clinical value for predicting cardiovascular events.

The present study indicates that in the setting of pregnant women with preexisting, or recently developed DCM during the initial phase of pregnancy, serial NTproBNP levels measurement, as opposed to serial echocardiography studies, may be a useful clinical tool for monitoring and managing these pregnant women. A rise in NTproBNP level (either early, or

particularly during the early delivery stage) appears to predict the occurrence of significant adverse events.

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References

1. Bernstein PS, Magriples U. Cardiomyopathy in pregnancy: a retrospective study. *Am J Perinatol* 2001; 18(3): 163-8.
2. van Hoeven KH, Kitsis RN, Katz SD, Factor SM. Peripartum versus idiopathic dilated cardiomyopathy in young women – a comparison of clinical, pathologic and prognostic features. *Int J Cardiol* 1993; 40(1): 57-65.
3. Hunter S, Robson SC. Adaptation of maternal heart in pregnancy. *Br Heart J* 1992: 68: 540-3.
4. Mabie WC, DiSessa TG, Crocker LG, et al. A longitudinal study of cardiac output in normal human pregnancy. *Am J Obstet Gynecol* 1994; 170: 849-56.
5. Vered Z, Poler SM, Gibson P, et al. Noninvasive detection of morphologic and hemodynamic changes during normal pregnancy. *Clin Cardiol* 1991; 14: 327-34.
6. Daniels LB, Maisel AS. Natriuretic peptides. *J Am Coll Cardiol* 2007; 50: 2357-68.
7. Amir O, Paz H, Ammar R, et al. Usefulness and predictive value of circulating NTproBNP levels to stratify patients for referral and priority treatment in a specialized outpatient heart failure center. *IMAJ Isr Med Assoc J* 2008; 10: 109-12.
8. Resnik JL, Hong CH, Resnik R, et al. Evaluation of B-type natriuretic peptide (BNP) levels in normal and preeclamptic women. *Am J Obstet Gynecol* 2005; 193: 450-4.
9. Bourdillon PVD, Broderick TM, Sawada SG, et al. Regional wall motion index for infarcted and noninfarcted regions after reperfusion in acute myocardial infarction: comparison with global wall motion index. *J Am Soc Echocardiogr* 1989; 2: 398-402.
10. Siu SC, Sermer M, Colman JM, et al. Prospective multicenter study of pregnancy outcomes in women with heart disease. *Circulation* 2001; 104: 515-21.
11. Felker GM, Thompson RE, Hare JM, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med* 2000; 342: 1077-84.
12. Elkayam U, Akhter MW, Singh H, et al. Pregnancy-associated cardiomyopathy: clinical characteristics and a comparison between early and late presentation. *Circulation* 2005; 111: 2050-5.
13. Ford RF, Barton JR, O'brien JM, Hollingsworth PW. Demographics, management, and outcome of peripartum cardiomyopathy in a community hospital. *Am J Obstet Gynecol* 2000; 182: 1036-8.
14. Redfield MM, Rodeheffer RJ, Jacobsen SJ, et al. Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Cardiol* 2002; 40: 976-82.

Capsule

Central roles of NLRs and inflammasomes in viral infection

The immune response to viral infections is determined by a complex interplay between the pathogen and the host. Innate immune cells express a set of cytosolic sensors to detect viral infection. Recognition by these sensors induces the production of type I interferons and the assembly of inflammasome complexes that activate caspase-1, leading to production of interleukin-1β (IL-1β) and IL-18. Kannegant discusses recent progress in our understanding of the central roles of NOD-like receptors (NLRs) and inflammasomes in the immune response during viral infections. This information will improve our understanding of host defense mechanisms against viruses and provide new avenues for interfering in the pathogenesis of infectious diseases.

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Eitan Israeli

Capsule

Mosquito malarial memory

During their life cycle malaria parasites produce vast numbers of successive proliferative stages in their vertebrate hosts, and yet in the field most mosquitoes are free of parasites. Rodrigues et al. report that the immune system of mosquitoes is primed early on when the malaria parasite (*Plasmodium* spp.) first crosses the mosquito gut epithelial barrier. A substantial (2- to 3.2-fold) increase in a single type of hemocyte (macrophage-like insect immune cells) is implicated in long-lived antiplasmodial immunity. This work may prove important for malaria control and for understanding immune memory in invertebrates.

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Eitan Israeli

"Men of genius are often dull and inert in society, as a blazing meteor when it descends to earth, is only a stone"

Henry Wadsworth Longfellow (1807-1882), American poet and educator

Kikuchi-Fujimoto Disease

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ABSTRACT: **Background:** Kikuchi-Fujimoto disease is a benign and self-limited disease, first reported in Japan in 1972. The characteristic features of this disorder include lymphadenopathy and fever.

Objectives: To summarize our experience with Kikuchi disease with regard to clinical manifestations and outcome.

Methods: The patients included in the study were those diagnosed with Kikuchi disease during the years 2005–2008 in two departments of internal medicine at Sheba Medical Center.

Results: We identified five patients with Kikuchi disease; four were women and the mean age was 22.6 years. All the patients had cervical lymphadenopathy; three had other sites of lymphadenopathy. Four of the patients had fever higher than 39ºC. Two of them had splenomegaly and three reported weight loss. Three of the five patients experienced a relapse of the disease and were treated with steroids or non-steroidal anti-inflammatory agents. The diagnosis was confirmed in all the patients by an excisional biopsy of lymph node.

Conclusions: Kikuchi disease must be considered in every young patient with fever and lymphadenopathy. The disease usually has a benign course.

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KEY WORDS: Kikuchi-Fujimoto disease, lymphadenopathy, fever, benign course, symptomatic treatment

Kikuchi-Fujimoto disease, also known as histiocytic necrotizing lymphadenitis, is a rare, benign and self-limited disorder that was first described in Japan in 1972 almost simultaneously by Kikuchi and Fujimoto [1,2]. The two most characteristic features of this disorder are regional tender lymphadenopathy (mostly cervical) and fever. Other findings include night sweats, weight loss, nausea, vomiting and sore throat [3]. The disease is more frequent among Asians, especially Japanese, and was thought to be much more common among women, with a female to male ratio of about 4:1. Recent reports, however, suggest that the actual ratio is closer to 1:1 [4,5]. Most of the patients are under the age of 30 [6]. The etiology is unknown, but some reports suggest an immunologic and infectious pathogenesis. Diagnosis is based on excisional biopsy of enlarged lymph nodes.

In this article we present five patients with Kikuchi disease who were diagnosed in our medical center. Their clinical course, laboratory data and radiologic evaluation are described. The pathologic findings, differential diagnosis and prognosis of this entity are discussed.

PATIENTS AND METHODS

A retrospective analysis of the records of the patients diagnosed with Kikuchi-Fujimoto disease in our departments of internal medicine during the years 2005 to 2008 was performed. The clinical presentation and the clinical course of the patients are described, as is the laboratory and radiologic evaluation.

PATIENT 1

The first patient was a 21 year old woman of Moroccan-Syrian origin who presented with fever of 6 weeks duration, up to 39ºC, accompanied by unilateral submandibular lymphadenopathy. She reported 5 kg weight loss over that period, abdominal pain, arthralgia and minimal complaints of sore throat. She was in contact with a friend who had infectious mononucleosis and had initially attributed her symptoms to Epstein-Barr virus.

On examination her vital signs were normal. She had enlarged tender left submandibular lymph nodes and enlarged and tender supraclavicular and axillary lymph nodes. The spleen was palpated 3 cm below the rib cage. Laboratory and radiologic evaluation are presented in Tables 1 and 2. A biopsy of supraclavicular lymph node was performed and Kikuchi's disease was diagnosed.

The patient was discharged on treatment with non-steroidal anti-inflammatory drugs and prednisone. Five days after prednisone cessation, there was a relapse of the cervical lymphadenopathy and fever. She was readmitted and was treated with solumedrol.

PATIENT 2

The second patient was a 20 year old man of Moroccan origin who presented with fever of 4 weeks duration, up to 39.5ºC, and unilateral cervical lymphadenopathy. He was treated by his family physician with amoxicillin and amoxicillin + clavulanic acid.

He reported losing 6 kg during that period. His weight at admission was 66 kg, so he lost 8.5% of his weight in one month. At admission, vital signs were normal. Palpation

Table 1. Laboratory evaluation of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
ESR	109	60	90	30	20
CRP	48.6			4.21	
WBC	4.6	3.3	6.4	5	5
Hb	12	12.5	12.2	12.2	11.5
LDH	174	338	178	215	
HIV	Negative		Negative	Negative	
anti-HA IgM	Negative				
HBsAg	Negative				
anti HCV	Negative				
CMV Ab IgM	Negative		Negative		
EBV EBNA Ab IgG	Positive				
EBV VCA Ab IgG	Positive				
EBV VCA Ab IgM	Negative	Negative			
EBV ELISA IgM	Positive		Negative		
EBV EBNA	Positive				
Blood culture	Negative	Negative	Negative		
ANF	Positive		Negative		
RF	Negative		Negative		
P&C ANCA			Negative		

ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, Hb = hemoglobin, WBC = white blood cells, LDH = lactate dehydrogenase, HIV = human immunodeficiency virus, Ig = immunoglobulin, HCV = hepatitis C virus, CMV = cytomegalovirus, EBV = Epstein-Barr virus, EBNA = Epstein-Barr nuclear antigen, VCA = viral capsid antigen, ELISA = enzyme-linked immunosorbent assay, ANF = antinuclear factor, RF = rheumatoid factor, ANCA = anti-neutrophil cytoplasmic antibodies, P = perinuclear, C = cytoplasmic

revealed bilateral cervical lymphadenopathy. An enlarged spleen was palpated 2 cm below the rib cage. Ear, nose and throat examination was normal. He underwent a lymph node biopsy of an enlarged cervical lymph node [Figures 1 and 2] and was diagnosed with Kikuchi's disease.

He was discharged from the hospital and was invited for follow-up as an outpatient. but was tragically killed in the second Lebanon war, before his appointment at the hospital outpatient clinic.

PATIENT 3

The third patient was a 23 year old woman of Russian origin who was admitted to hospital due to fever, up to 40ºC, of 2 weeks duration, cervical lymphadenopathy and weight loss. Two months earlier she returned from a trip to India. A week before admission she suffered from abdominal pain and diarrhea. Her history was vague regarding a diagnosis of celiac disease.

On admission her vital signs were normal, except for tachycardia (100 beats/min). She had mildly enlarged cervical lymphadenopathy and small axillary lymph nodes. Serology tests for West Nile fever, Dengue, Toxoplasma, Rubella, Borrelia, and Brucella were all negative. Due to her so-called celiac disease, and suspected lymphoma secondary to celiac disease, a laparoscopic biopsy of two lymph nodes at the mesentery of the terminal ileum and a lymph node from the hilum of the liver was performed. The diagnosis of Kikuchi was established from the lymph nodes.

One year later she had a relapse of the disease, with high fever up to 40°C of 2 weeks duration and weight loss of 3 kg, 1 month after returning from Thailand. On physical examination, cervical lymphadenopathy was found, and abdominal ultrasonography demonstrated enlarged lymph nodes. Some of them were suspected to be necrotic. She was treated with prednisone which led to a rapid improvement.

PATIENT 4

The fourth patient was a 34 year old woman of Sephardic origin who presented with unilateral cervical lymphadenopathy of 10 months duration that had become larger and tender over the previous 2 months. At that time she noticed additional enlarged lymph nodes on the same side of her neck. A week prior to her admission she had fever, up to 39.7ºC. She reported having arthralgia of her right wrist and both shoulders. She was treated in the health fund clinic with amoxicillin + clavulanic acid, with no improvement.

Table 2. Radiologic evaluation of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Chest X-rays	Normal	Normal, except for swelling at the left side of the neck	Normal	Normal	
Abdominal ultrasound	Enlarged spleen (13 cm)	Enlarged lymph nodes at the upper retroperitoneum: at the gastrohepatic ligament, portal hilum	Two areas of lymph nodes demonstrated at the retroperitoneum, and the hilum of the liver	ND	
Neck ultrasound	ND	ND	ND	A collection of enlarged, vascular, hypoechogenic lymph nodes on the right side	
CT	A few lymph nodes at the retroperitoneum and the base of the neck	A lymph node near the inferior vena cava at the retroperitoneum. Neck – multiple lymph nodes with fat infiltration	Enlarged lymph nodes at the upper retroperitoneum: at the gastrohepatic ligament, portal hilum, and at the mesenterium	Enlarged lymph nodes at the right side of the neck	Sub-auricular, bilateral lymphadenopathy

Figure 1. Hematoxylin & eosin x 200. Area of necrosis (upper left) surrounded by cellular infiltrates and numerous cellular debris and nuclear dust.

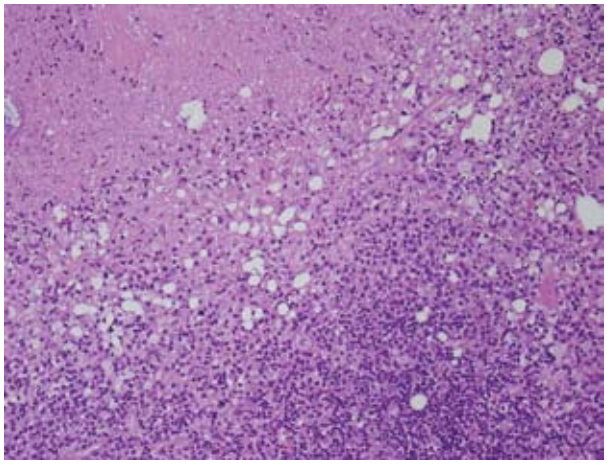
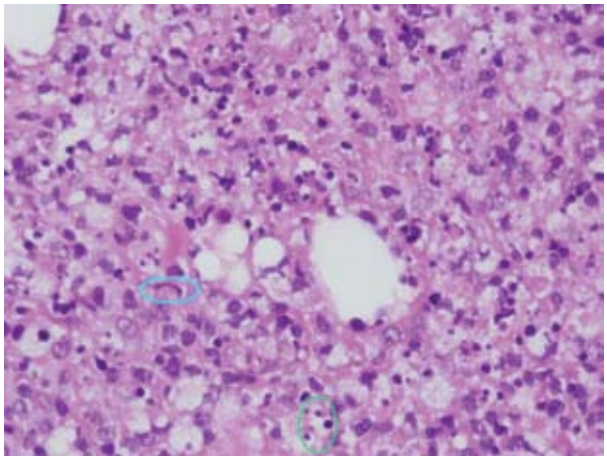


Figure 2. H&E x 600. Cell infiltrates composed of pale-staining histiocytes, some of them with "crescentic" nuclei resembling signet-ring cells (marked by horizontal blue ellipse), small lymphocytes, immunoblasts and numerous apoptotic cells and nuclear dust (marked by vertical green ellipse). No granulocytes or eosinophils are demonstrated.



On admission her vital signs were normal, except for tachycardia (110 bpm). She had tender cervical lymphadenopathy. A biopsy of an enlarged lymph node was performed and Kikuchi disease was diagnosed. The fever and arthralgia resolved after treatment with antipyretic drugs and she became asymptomatic.

PATIENT 5

The last patient was a 15 year old girl who presented with right cervical lymphadenopathy without fever. She was treated by her family physician with augmentin, but there was no improvement. Palpation revealed right cervical lymphadenopathy of about 3 cm. Ear, nose and throat examination was normal. Serology for cytomegalovirus and Toxoplasma were suggestive of past illness. She was treated with clindamycin but there was no improvement. She underwent a lymph node biopsy and was diagnosed with Kikuchi disease.

During the following 3 years she presented repeatedly with bilateral submandibular lymphadenopathy, headaches and fever, and was treated with NSAIDs. She later became asymptomatic.

DISCUSSION

Kikuchi disease is known to have a worldwide distribution with higher prevalence among Japanese and other Asiatic people. Kikuchi disease among Israelis was described recently by Rimar et al. [7]. In this article we describe the patients who were diagnosed and treated in two departments of internal medicine in Sheba Medical Center during a 4 year period (2005–2008).

Age at diagnosis in our patients ranged from 15 to 34 years, with a mean age of 22.6. There is a definite trend of presentation at young age, as demonstrated in many of the published series. In their work of 108 cases of Kikuchi disease, Dorfman and Berry [6] reported a mean age of 30 (range 11–75). Kucukardali et al. [8] reported a mean age of 25 (range 1–64) in their research of 244 cases, and Lin and co-authors [4] reported a mean age of 21 (range 6–46).

Four of our patients were women. Earlier reports of Kikuchi disease described a female predominance of 4:1. In recent reports, however, the ratio is closer to 1:1 with a slight female predominance [4-7].

Lymph node enlargement is an important manifestation of Kikuchi disease; the majority of cases present with cervical and unilateral lymphadenopathy [5,6,8,9]. Only 3 of 108 cases reported by Dorfman and Berry [6], and 7 of the 61 cases reported by Lin et al. [4] had bilateral cervical lymphadenopathy. All of our patients presented with enlarged cervical lymph nodes. Four of the patients had unilateral lymphadenopathy, and one had bilateral cervical lymphadenopathy. Other sites of lymphadenopathy reported in the literature are axillary, supraclavicular, mediastinal, inguinal, intraparotid, celiac, peripancreatic and retroperitoneal – all in sporadic patients [6- 9]. Three of our patients had lymphadenopathy that involved lymph nodes other than cervical. The dimension of the lymph nodes in our study ranged from smaller than 1 cm to 2–4 cm. In other publications lymph nodes ranged from 0.5 to 9 cm, were rarely larger than 6 cm [3], and 75% were < 2 cm [5].

Fever is another important manifestation of Kikuchi disease. It was reported in about 30–40% of patients [4-6]. In the

NSAID = non-steroidal anti-inflammatory drug

Israeli series described by Rimar et al. [7], 73% of the patients had fever. Four of our patients had fever higher than 39ºC.

Two of our patients had splenomegaly on physical examination and imaging evaluation. Weight loss is reported in 5–9% of Kikuchi patients [6,8]; 3 of our patients reported weight loss prior to their diagnosis. Two of our patients reported arthralgia. Reports in the medical literature are of a smaller scale, ranging from 4 to 7% [6,8].

Laboratory investigation is usually unremarkable and less suggestive for establishing a diagnosis of Kikuchi disease, but negative results might help to exclude other conditions. One of our patients had leukopenia, which is considered one of the most common laboratory findings among Kikuchi patients and ranges from 23% to 58% [5,9].

One patient had positive antinuclear antibodies and was later diagnosed with mild systemic lupus erythematosus.

In our series three patients (60%) had a relapse of the disease. Such a high rate of recurrent disease is unusual, and recurrent Kikuchi disease is estimated to occur in about 3% of patients [7,10]. The explanation for this finding is not clear.

The etiology and pathogenesis of Kikuchi disease are also not clear. Various infections have been postulated to be the cause. Most studies raise the possibility of immune system involvement. Apoptotic cell death appears to be the principal finding in the histogenesis of this disease [11]. The recurrent disease in the third patient and the many patients with Kikuchi disease described in the Far East raise the possibility of an unknown infectious agent as the causative agent. The diagnosis of this disease is done by excisional biopsy of affected lymph nodes.

Although Kikuchi is a rare disease, it should be considered in the differential diagnosis of "lymph node enlargement." Its course, treatment and follow-up differ from most of the other diseases on that list. The differential diagnosis of Kikuchi disease includes lymphoma, tuberculosis, reactive lesions such as lymphadenitis associated with SLE or herpes simplex, non-Hodgkin's lymphoma, Kawasaki's disease, and metastatic adenocarcinoma [3,9]. The main diagnostic challenge is that lymphoma can be easily confused with Kikuchi [12]. It is important to distinguish one from the other because the course, treatment and prognosis of these disorders differ dramatically. It is assumed that some of the cases that were diagnosed as malignant lymphoma were actually more consistent with Kikuchi [2,14], and there are reports of patients receiving cytotoxic therapy for no apparent reason [14-16]. While there are histologic and immunohistochemical methods to differentiate between these two disorders, the clinician's awareness of Kikuchi disease is very important.

Distinguishing Kikuchi from SLE is sometimes problematic. Both entities share similar presenting symptoms and histologic characteristics. Furthermore, there is a well-known and documented relationship between these two entities [5,8,9].

Kikuchi disease has a benign course and is self-limited usually within 6 months from diagnosis [8]; it usually requires no specific treatment. Symptomatic treatment measures such as analgesics, antipyretics, etc. are used to relieve patients' complaints [9]. Severe cases may be treated with corticosteroids [8,17-19]. There is also a report on treatment with chloroquine and hydroxychloroquine [20] and intravenous immunoglobulin was also used effectively to treat a patient with severe Kikuchi disease [21]. It is important for patients with Kikuchi to undergo long-term follow-up, mainly for assessing and evaluating development of SLE and prompt detection of recurrences.

In summary, we present five diverse cases that demonstrate the wide range of clinical and laboratory presentation of Kikuchi disease. All cases were diagnosed in our institute over 4 years. This series and the cases described recently by Rimar and colleagues [7] suggest that only the combination of experienced pathologists, but primarily the awareness of clinicians, may lead to the prompt diagnosis of Kikuchi disease. The disease is probably more common than was previously believed in Israel.

SLE = systemic lupus erythematosus

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References

1. Kikuchi M. Lymphadenitis showing focal reticulum cell hyperplasia with nuclear debris and phagocytes: a clinicopathological study. *Acta Hematol Jpn. Nippon Ketsueki Gakkai Zasshi* 1972; 35: 379-80.
2. Fujimoto Y, Kjima Y, Yamaguchi K. Cervical subacute necrotizing lymphadenitis: a new clinicopathologic entity. *Naika* 1972; 20: 920-7.
3. Bosch X, Guilabert A, Miquel R, Campo E. Enigmatic Kikuchi-Fujimoto disease: a comprehensive review. *Am J Clin Pathol* 2004; 122: 141-52.
4. Lin HC, Su CY, Huang CC, Hwang CF, Chien CY. Kikuchi's disease: a review and analysis of 61 cases. *Otolaryngol Head Neck Surg* 2003; 128: 650-3.
5. Yu HL, Lee SS, Tsai HC, et al. Clinical manifestations of Kikuchi's disease in southern Taiwan. *J Microbiol Immunol Infect* 2005; 38: 35-40.
6. Dorfman RF, Berry GJ. Kikuchi's histiocytic necrotizing lymphadenitis: an analysis of 108 cases with emphasis on differential diagnosis. *Semin Diagn Pathol* 1988; 5: 329-45.
7. Rimar D, Zisman D, Schendler Y, et al. Kikuchi-Fujimoto disease in Israel – more than pain in the neck. *Semin Arthritis Rheum* 2010; 39: 515-20.
8. Kucukardali Y, Solmazgul E, Kunter E, Oncul O, Yildirim S, Kaplan M. Kikuchi-Fujimoto disease: analysis of 244 cases. *Clin Rheumatol* 2007; 26: 50-4.
9. Bosch X, Guilabert A, Miquel R, Campo E. Enigmatic Kikuchi-Fujimoto disease: a comprehensive review. *Am J Clin Pathol* 2004; 122: 141-52.
10. Kuo TT. Kikuchi's disease (histiocytic necrotizing lymphadenitis). A clinicopathologic study of 79 cases with an analysis of histologic subtypes, immunohistology, and DNA ploidy. *Am J Surg Pathol* 1995; 19: 798-809.
11. Iguchi H, Sunami K, Yamane H, et al. Apoptotic cell death in Kikuchi's disease: a TEM study. *Acta Otolaryngol Suppl* 1998; 538: 250-3.
12. Chamulak GA, Brynes RK, Nathwani BN. Kikuchi-Fujimoto disease

mimicking malignant lymphoma. *Am J Surg Pathol* 1990; 14: 514-23.

13. Turner RR, Martin J, Dorfman RF. Necrotizing lymphadenitis. A study of 30 cases. *Am J Surg Pathol* 1983; 7: 115-23.
14. Dorfman RF. Histiocytic necrotizing lymphadenitis of Kikuchi and Fujimoto. *Arch Pathol Lab Med* 1987; 111: 1026-9.
15. Lerosey Y, Lecler-Scarcella V, Francois A, Guitrancourt JA. A pseudo-tumoral form of Kikuchi's disease in children: a case report and review of the literature. *Int J Pediatr Otorhinolaryngol* 1998; 45: 1-6.
16. Dylewski J, Berry G, Pham-Dang H. An unusual cause of cervical lymphadenitis: Kikuchi-Fujimoto disease. *Rev Infect Dis* 1991; 13: 823-5.
17. Bhat NA, Hock YL, Turner NO, et al. Kikuchi's disease of the neck (histiocytic necrotizing lymphadenitis). *J Laryngol Otol* 1998; 112: 898-900.
18. Martínez-Vázquez C, Hughes G, Bordon J, et al. Histiocytic necrotizing lymphadenitis, Kikuchi-Fujimoto's disease, associated with systemic lupus erythemotosus. *QJM* 1997; 90: 531-3.
19. Jang YJ, Park KH, Seok HJ. Management of Kikuchi's disease using glucocorticoid. *J Laryngol Otol* 2000; 114: 709-11.
20. Rezai K, Kuchipudi S, Chundi V, Ariga R, Loew J, Sha BE. Kikuchi-Fujimoto disease: hydroxychloroquine as a treatment. *Clin Infect Dis* 2004; 39: e124-6.
21. Noursaeghi M, Aqel N, Gibson P, Pasvol G. Successful treatment of severe Kikuchi's disease with intravenous immunoglobulin. *Rheumatology* 2006; 45: 235-7.

Capsule

Damaged intestinal epithelial integrity linked to microbial translocation in SIV infection

Estes and co-researchers used monoclonal antibodies specific for the core antigen of lipopolysaccharide (LPS) to stain colon tissue sections obtained from necropsies of SIV-infected rhesus macaques that were euthanized at different phases of infection, including early acute infection and chronic infection, as well as sections from uninfected controls. In the uninfected animals and during early acute infection there was abundant staining for LPS in the intestinal lumen but little staining in the lamina propria of the large bowel. By contrast, in chronically infected animals there was abundant staining for intracellular and extracellular LPS in the lamina propria. Abundant LPS staining was also seen in the mesenteric and axillary lymph nodes and the livers of chronically infected animals, but not in those of uninfected animals or during early acute infection. Moreover, staining for the tight junction component claudin 3 showed that the integrity of the epithelial barrier was severely compromised in chronically infected animals. This damage began during the late acute phase of infection, and the degree of damage correlated with the degree of LPS translocation. The authors then investigated whether this translocation was linked to immune activation. Using double-label immunohistochemical staining, they found colocalization of LPS and the proinflammatory mediators interferon-α and interleukin-18 in chronically infected animals. Importantly, both mediators could be observed in close proximity to LPS rather than to sites of local SIV replication. Confocal microscopy also revealed that macrophages were abundant in gastrointestinal tissue during the chronic stage of infection but that a high proportion of these had not taken up any LPS, suggesting that macrophage-based clearance is impaired. Taken together, these data provide direct evidence to support the hypothesis that the systemic immune activation that occurs during chronic HIV-1 infection is linked to the translocation of microbial products from the intestinal lumen, which in turn is linked to damage to the structural integrity of the gut epithelial layer and impaired clearance by macrophages.

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Eitan Israeli

Capsule

CRISPR Processing of invading viruses

Many bacteria and archaea recognize invading viruses and plasmids. Foreign DNA is integrated into so-called clustered regularly interspaced short palindromic repeat (CRISPR) loci, and transcripts from these loci are processed into RNAs that can target the invading DNA or RNA for destruction. To investigate the molecular basis for this processing, Haurwitz and team screened CRISPR-associated (Cas) proteins in the opportunistic pathogen *Pseudomonas aeruginosa* and found they were capable of cleaving the CRISPR transcripts. The crystal structure of Cas4 with the CRISPR RNA transcript revealed how the protein specifically recognized RNA repeats, as well as the mechanism of endonucleolytic cleavage.

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Eitan Israeli

"From a very early age I've had to interrupt my education to go to school"

George Bernard Shaw (1856-1950), Irish playwright, a co-founder of the London School of Economics, and Nobel Peace Prize laureate. Nearly all his writings 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. An ardent socialist, he was most angered by what he perceived as the exploitation of the working class.

Predictors of Pandemic (H1N1) 2009 Virus Positivity and Adverse Outcomes among Hospitalized Patients with a Compatible Syndrome

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ABSTRACT: **Background:** A pandemic (H1N1) influenza A virus was identified in 2009.

Objectives: To investigate predictors for pandemic (H1N1) 2009 virus infection among hospitalized patients with a flu-like illness and to identify parameters suggesting a severe clinical course.

Methods: We analyzed a cohort of all patients hospitalized during a 2 month period with a flu-like syndrome who were tested for pandemic (H1N1) 2009 infection. Demographic, clinical and laboratory, along with outcome parameters, were recorded and compared between pandemic (H1N1) 2009 virus-positive and negative hospitalized patients.

Results: Of the 179 examined hospitalized patients suspected of having pandemic (H1N1) 2009 infection 65 (36%) were found positive. These patients tended to be younger and had significantly fewer comorbidities. In addition, they had a significantly higher frequency of fever (94%), cough (86%) and myalgia (29%). Furthermore, age < 65 years and cough were independent predictors for pandemic (H1N1) 2009 virus positivity in a multivariate regression analysis. Notably, 14 of the 65 positive patients (21.5%) had acute respiratory insufficiency requiring treatment in the intensive care unit. These patients were neither older nor previously sicker than patients with non-severe disease, but were distinguished by augmented inflammatory markers, significant lymphopenia associated with disease severity, and overall mortality of 21.4%.

Conclusions: Pandemic (H1N1) 2009 virus-positive hospitalized patients tend to be younger and have fewer comorbidities as compared to compatible negative patients. A significant number of relatively young and previously healthy positive patients might develop severe disease associated with a robust inflammatory reaction and significant lymphopenia.

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KEY WORDS: pandemic (H1N1) 2009 virus, prognosis, mortality, intensive care unit

In late March 2009, an outbreak of respiratory illness occurred in Mexico that was later identified as a novel pandemic (H1N1) 2009 virus-related disease [1,2]. The first reports from Mexico of severe illness accompanied by respiratory failure and death among young previously healthy people infected with this virus suggested that this infection is a potentially serious health concern [3,4]. Accordingly, reports about the rapid spread of the virus to many countries outside of Mexico brought the World Health Organization to classify the global spread of the virus as an international concern with a pandemic level of six [5].

The lack of adequate data on the natural history of pandemic (H1N1) 2009 virus infection outside of Mexico led to uncertainty in approach to this new pandemic in the medical communities of many countries around the world. Furthermore, the first reports outside of Mexico of an unusual susceptibility of younger and previously healthy people to severe respiratory complications due to this virus [6-8] led to an ambivalence in approach to young patients with signs and symptoms of influenza [9]. Therefore, one of the major challenges in the combat with the H1N1 pandemic today is to identify patients presenting with flu-like illness who are more likely to be infected with this virus, and to identify the parameters predicting severe morbidity and mortality.

We present a case series of all consecutive patients hospitalized in a major medical center in Israel during a 2 month period with a presumed diagnosis of pandemic (H1N1) 2009 virus infection. Demographic, clinical and laboratory, along with outcome parameters, were analyzed in order to identify possible factors that may suggest the diagnosis of pandemic (H1N1) 2009 virus infection among patients with a flu-like illness, and furthermore, to examine which parameters can predict a more severe clinical course among these hospitalized patients.

PATIENTS AND METHODS

This study was conducted in the Tel Aviv Sourasky Medical Center, the major community and tertiary care university

* Both authors contributed equally to the study and manuscript

hospital in Tel Aviv (1150 beds), serving a population of approximately 750,000 people. The study population comprised all consecutive patients (n=179), including children, who were hospitalized during the 2 month period of the analysis (July–August 2009) and tested for the presence of pandemic (H1N1) 2009 infection based on clinical suspicion. The decision to hospitalize suspected patients was based on comorbidity and symptom severity, hypoxia or radiographic evidence of pneumonia. The data of the admitted 179 patients were collected and analyzed.

Treatment with oseltamivir was initiated in virtually all hospitalized patients with suspected pandemic (H1N1) 2009 virus infection. Treatment was stopped upon validation of the patient's negativity for this infection based on the reverse transcriptase-polymerase chain reaction test.

DATA COLLECTION AND STUDY DESIGN

Demographic, clinical and laboratory data were obtained by retrospectively reviewing patients' medical records. The study was designed as a case series of all the 179 patients tested for pandemic (H1N1) 2009 infection, with subsequent comparison between the virus-positive and negative groups, and further analyzing the positive patients for difference between those with and without a severe clinical course. The study was approved by the hospital's institutional review board. Comorbidities were rated according to the Charlson index, a validated prognostic comorbidity score that takes into account the number and severity of comorbid diseases, with higher scores representing more severe comorbidities [10].

PANDEMIC (H1N1) 2009 VIRUS TEST

Nasal and throat viral swabs (a total of three) were obtained from suspected patients and were transferred refrigerated to the Israeli reference laboratory for RT-PCR testing according to WHO recommendations.

STATISTICAL ANALYSIS

Analyses, using Stata version 9 (Stata Corp., College Station, TX, USA), were performed with respect to the main study aim: predicting H1N1 positivity among hospitalized tested patients. Student's *t*-test was used to compare normally distributed continuous variables, Wilcoxon rank sum test for non-normally distributed variables. Chi-square test was used to analyze dichotomized variables. Multivariable analysis was performed using logistic regression. All variables with a P value < 0.1 were included in the models, and those with $P \leq 0.05$ were retained in the model. Area under the ROC curve was calculated to estimate the model's ability to predict positivity. $P \leq 0.05$ was considered statistically significant.

RT-PCR = reverse transcriptase-polymerase chain reaction
WHO = World Health Organization

RESULTS

DEMOGRAPHIC, CLINICAL AND LABORATORY PARAMETERS

During the 2 month period July to August 2009, 179 patients admitted to our hospital were tested for the presence of S-OIV (swine-originated influenza virus), based on the presence of signs and symptoms of upper respiratory tract infection. Treatment with oseltamivir was initiated in almost all patients, but only 65 of the 179 (36%) were ultimately found to be positive for pandemic (H1N1) 2009 infection.

As shown in Table 1, H1N1 virus-positive patients tended to be younger with significantly fewer comorbidities, as compared to negative patients. In addition, typical signs and symptoms of flu-like illness such as fever, cough and myalgia were significantly more common among H1N1-positive patients [Table 1].

Table 1. Demographic, clinical and laboratory characteristics of admitted patients tested for the presence of pandemic (H1N1) 2009 virus infection

Variable	S-OIV positive (N=65)	S-OIV negative (N=114)	*P* value
Gender (%, Male/Female)	55.4/44.6	57.9/42.1	0.74
Age (yrs, mean ± SD)	37 ± 21.7	44.9 ± 29.8	0.06
Comorbidity score (mean ± SD)*	1.26 ± 1.72	2.38 ± 2.45	0.001
Time to S-OIV sample**	1.31 ± 3.2	2.71 ± 5	0.045
Samples on first day (%)	76.9	57	0.007
Symptoms [No. of patients (%)]			
Fever	61 (93.8)	86 (75.4)	0.002
Cough	56 (86.2)	54 (47.4)	< 0.0001
Dyspnea	22 (33.9)	47 (41.2)	0.33
Myalgia	19 (29.2)	17 (14.9)	0.021
Sore throat	12 (18.5)	16 (14)	0.4
Abdominal pain	2 (3.1)	10 (8.8)	0.14
Vomiting	15 (23.1)	18 (15.8)	0.22
Diarrhea	12 (18.5)	12 (10.5)	0.13
Fever+cough+myalgia	16 (24.6)	12 (10.5)	0.013
Laboratory values [Median (IQR*)]			
BUN_{max} (mg/dl)	14 (10-24)	18 (11-40)	0.06
LDH_{max}(IU/L)	501 (360-792)	538 (399-865)	0.19
ALT_{max}(U/L)	32 (23-67)	33 (21-76)	0.9
CK_{max}(IU/L)	131 (74-654)	94 (65-287)	0.019
$Lymphocytes_{min}$ ($x10^3/\mu l$)	0.8 (0.5-1.32)	0.8 (0.4-1.4)	0.74
WBC_{max} ($x10^3/\mu l$)	9.4 (7.15-13.7)	9.8 (8.1-18.2)	0.14
$Platelets_{max}$ ($x10^3/\mu l$)	269 (225-422)	319 (242-508)	0.09
CRP_{max} (mg/dl)	58 (30-153)	65 (20-149)	0.9

* Charlson comorbidity score was calculated as described in Methods.

** Days from admission

S-OIV = swine-originated influenza virus, IQR = interquartile range, CRP = C-reactive protein, LDH = lactate dehydrogenase, ALT = alanine aminotransferase, CK = creatine kinase.

However, no significant difference in major laboratory values, except for a slight rise in the level of creatine kinase was detected between the virus-positive and negative patients [Table 1].

Multivariable logistic regression identified two variables as important independent predictors of H1N1 positivity: age younger than 65 (odds ratio 12.5, $P < 0.0001$) and the presence of cough (OR 5.9, $P < 0.0001$). The model had an area under the ROC curve of 0.81, suggesting a very good prediction.

Interestingly, the mean time from admission to the first sampling for the presence of pandemic (H1N1) 2009 virus was significantly shorter among the virus-positive patients, reflecting the much higher percentage of patients among the positive patients for whom a H1N1 virus sample was taken on admission: 77% in the virus-positive group versus 57% in the negative group.

OR = odds ratio

Table 2. Comparison of clinical outcome parameters during hospitalization between pandemic (H1N1) 2009 virus-positive and negative patients

Variable	S-OIV positive (n=65)	S-OIV negative (n=114)	*P* value
Duration of hospitalization in patients suspected on admission (days, mean ± SD)	3 ± 2.5	4.1 ± 3.7	0.12
Pneumonia	28 (43.1%)	43 (37.7%)	0.5
Mechanical ventilation	9 (13.8%)	12 (10.5%)	0.5
Transfer to ICU	14 (21.5%)	16 (14%)	0.2
Death	3 (4.6%)	8 (7%)	0.5

Table 3. Comparison of demographic, clinical and laboratory characteristics of ICU vs. non-ICU pandemic (H1N1) 2009 virus-positive patients

Variable	ICU (n=14)	Non-ICU (n=51)	*P* value
Demographic			
Age (yrs, mean ± SD)	35.4 ± 23.4	37.5 ± 21.5	0.7
Gender (%, Male/Female)	42.9/57.1	58.8/41.2	0.29
Co-morbidity score (mean ± SD)*	0.86 ± 1.83	1.37 ± 1.68	0.3
Laboratory data [Median (IQR)]			
BUN_{max} (mg/dl)	25 (11-58)	13 (9-18)	0.01
LDH_{max}(IU/L)	833 (607-1096)	404 (305-531)	< 0.0001
ALT_{max}(U/L)	135 (39-178)	28 (21-46)	0.0001
CK_{max}(IU/L)	922 (90-1945)	117 (73-416)	0.027
$Lymphocytes_{min}$ (x10^3/μl)	0.5 (0.2-0.725)	0.9 (0.625-1.47)	0.0029
WBC_{max} (x10^3/μl)	14.9 (10.5-22)	8.8 (6.8-11.5)	0.002
$Platelets_{max}$ (x10^3/μl)	458 (262-724)	250 (207-328)	0.004
CRP_{max} (mg/dl)	165 (105-196)	39 (23-87)	0.0001
Outcome			
Duration of hospitalization in patients suspected on admission (days, mean ± SD)	5.6 ± 2.7	2.6 ± 2.2	0.0026
Pneumonia	14 (100%)	14 (27.5%)	< 0.0001
Mechanical ventilation	9 (64%)	0 (0)	< 0.0001
Death	3 (21)	0 (0)	0.0007

* Charlson comorbidity score was calculated as described in Methods.

CLINICAL OUTCOMES

Reports from Mexico, where the current pandemic originated, suggest that the new pandemic (H1N1) 2009 virus has the potential to cause a severe illness with life-threatening respiratory complications, especially among young previously healthy people [1,2]. To determine whether this is also the case in our study population we analyzed major clinical outcomes of patients who were tested for the presence of the virus, and compared those outcomes between the virus-positive and negative patients. Interestingly, although H1N1 virus-positive patients had fewer comorbidities and tended to be younger than the negative patients [Table 1], the two groups of patients were comparable in the occurrence of pneumonia, a well-known complication of influenza in general and of the pandemic (H1N1) 2009 virus strain in particular [Table 2]. Furthermore, there was no difference in the length of hospital stay or in the rate of transfer to the ICU, mechanical ventilation or death between the two groups of patients, despite the difference in age and background comorbidity, suggesting that pandemic (H1N1) 2009 infection tends to be a relatively serious disease in a significant proportion of hospitalized patients.

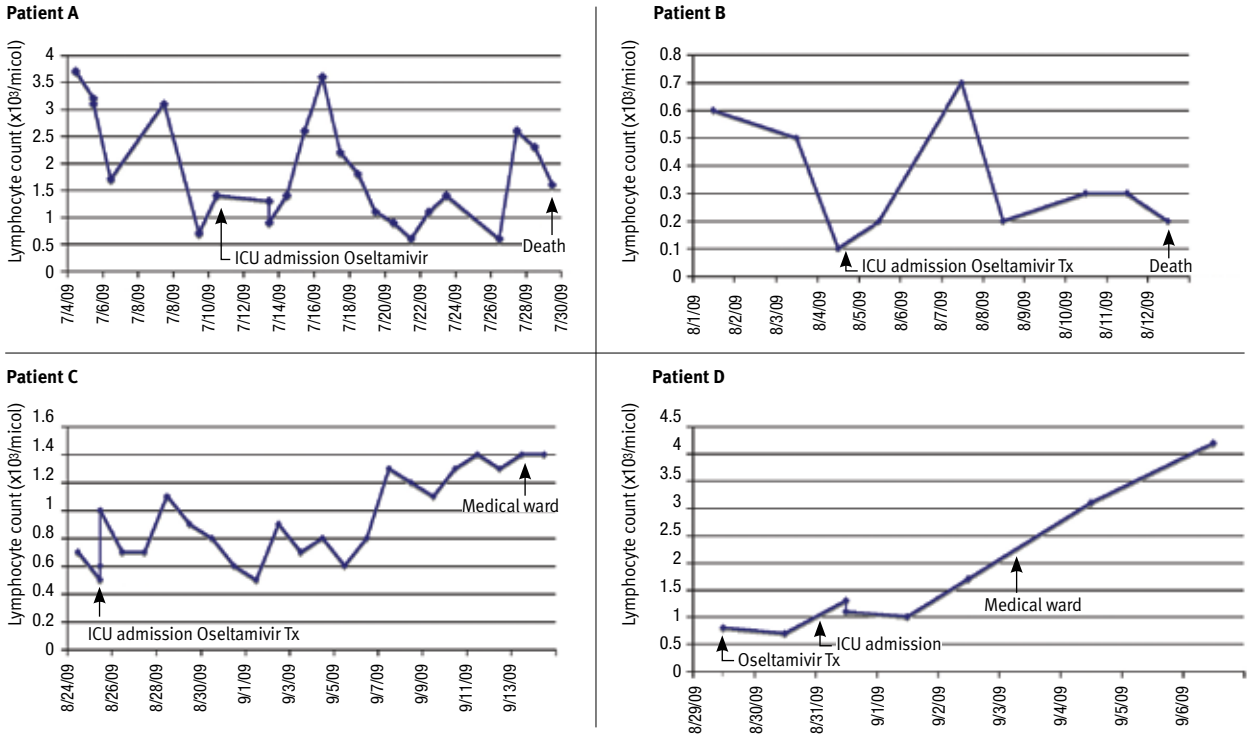
SEVERE DISEASE AND NON-SEVERE DISEASE

Of the 65 H1N1-positive patients 14 (21.5%) were transferred to the ICU during their hospitalization, most of them with respiratory failure that reflected a much more severe clinical course. We analyzed demographic, clinical and laboratory parameters of these patients and compared them to those of the infected patients who experienced a milder clinical course that did not necessitate a transfer to the ICU. As shown in Table 3, H1N1-positive patients with a severe clinical course were neither older nor did they have more comorbidities, as compared to virus-positive patients who experienced a milder disease. However, the complicated cases were distinguished by significantly higher indices of inflammation, such as leukocytosis, thrombocytosis and elevated C-reactive protein, and by much higher blood urea nitrogen, lactate dehydrogenase, alanine aminotransferase and creatine kinase. Noteworthy is the significant severe lymphopenia observed among the ICU patients, compatible with previous reports from Mexico about lymphopenia accompanying severe pandemic (H1N1) 2009 infection [4].

As expected, clinical outcomes were worse among the ICU patients and the length of their hospital stay was significantly longer, with higher rates of pulmonary complications that

ICU = intensive care unit

Figure 1. Lymphopenia among ICU-admitted pandemic (H1N1) 2009 virus-positive patients correlates with disease severity. Graphic presentations of the lymphocyte counts of four representative ICU-admitted virus-positive patients (patients A-D) along their hospital course. Times of oseltamivir initiation, transfer to the ICU, readmission to the medical ward and death are indicated.



necessitated mechanical ventilation. Notably, pulmonary complications were the hallmark of a severe clinical course, reflected by the high percentage (50%) of patients with pulmonary infiltrates who developed respiratory failure and were transferred to the ICU. Consequently, 3 of the 14 ICU patients (21.4%) ultimately succumbed to their disease, whereas no cases of death occurred among the non-ICU pandemic (H1N1) 2009 virus-infected patients [Table 3].

CORRELATION BETWEEN LYMPHOPENIA AND DISEASE PROGRESSION

As noted above, pandemic (H1N1) 2009-infected patients with a severe clinical course, as defined by the need for their transfer to the ICU, were significantly more lymphopenic than non-ICU patients [Table 3]. The correlation between the lymphocyte count and the clinical course of the disease among virus-infected complicated patients is depicted in Figure 1. All four representative patients had significant lymphopenia that was at its nadir level around the time of their transfer to the ICU, correlating with the time of clinical deterioration and signs and symptoms of respiratory failure. Interestingly, initiation of treatment with oseltamivir led to a rise in the lymphocyte counts in all patients. However, whereas the lymphocyte counts of patients C and D continued to increase steadily correlating with the patients' recovery, patients A and B experienced an initial response in terms of lymphocyte counts that was immediately abrogated, associated with clinical deterioration and death. These observations suggest that lymphopenia is associated with a severe clinical course among pandemic (H1N1) 2009 virus-infected patients and its resolution tends to correlate with clinical recovery.

DISCUSSION

The emergent pandemic (H1N1) 2009 virus is challenging to the medical community around the world because of its unusual rapidity of spread and its tendency to complications among relatively young and previously healthy patients, seemingly considered a low risk group for influenza-induced complications [3,4,11]. However, despite increasing epidemiologic reports from countries outside of Mexico to which the virus has spread [6-8], it is still difficult to predict the natural course of the disease and establish strict policies for health care providers. The current study, which is based on a case series of H1N1-infected patients, seems to substantiate current knowledge on the natural history of the disease and also suggests predictors for both pandemic (H1N1) 2009 virus positivity among hospitalized patients with a flu-like syndrome and for a more severe clinical course among infected patients.

Several major conclusions may be drawn from the present analysis. First, among hospitalized patients suspected of having H1N1 infection, those found to be positive for the virus tend to be younger and have significantly fewer comorbidities as compared to those who are negative. Second, our study clearly shows that the typical symptoms and signs of influenza, including fever, cough and myalgia, are more common among pandemic (H1N1) 2009 virus-positive patients. Thus, supported by a multivariable logistic regression analysis of our data, we conclude that younger age and the presence of cough on presentation are two parameters that may predict H1N1 positivity among hospitalized patients with a flu-like illness. Obviously, the younger age of the positive patients can be explained by other medical conditions that can present with respiratory symptoms and are much more prevalent among older patients with comorbidities. Indeed, the observation that the pandemic (H1N1) 2009 virus-negative patients were tested for the presence of the virus later during their hospitalization course as compared to the positive patients reflects the ambiguity in the differential diagnosis of these patients' clinical presentation.

Most importantly, a significant number of infected patients were prone to severe complications and respiratory failure that necessitated an urgent transfer to the intensive care unit. These patients were distinctive for the presence of increased inflammatory markers. This observation may correlate with immune system over-reaction to the viral infection, leading to the so-called cytokine storm, which triggers inflammation and lung damage that can lead to multiple organ failure and death [12,13].

Interestingly, in contrast to the typical seasonal flu that tends to complications in older and debilitated patients [14], the severely ill H1N1-infected patients were neither older nor previously sicker than the H1N1-infected patients with a milder clinical course. This observation joins a growing body of evidence indicating that the novel pandemic (H1N1) 2009 virus tends to complications in relatively young people who were traditionally thought to be at a low risk for flu-related complications [1-4,6-8,11]. Recent evidence suggests that the younger age of the severely infected patients may be due to the presence of cross-reacting protective antibodies found in older individuals who have been exposed to the Spanish flu virus. These antibodies cross-react with the present pandemic (H1N1) 2009 virus, providing the elderly population a relative protection from the present epidemic [15]. However, in contrast to recent studies suggesting a severe clinical course in patients with at least one significant comorbidity [6-8], the patients with a severe clinical course in our study had no more comorbidities than patients with a non-severe clinical course. However, the three cases of deaths had significant comorbidities (one patient after bone marrow transplantation, one with metastatic colon cancer and one with a history of alcohol abuse and intavenous drug use) that apparently led to the fatal outcome.

The present analysis has several limitations, mainly its retrospective nature and its restriction to hospitalized patients. In addition, some of the admitted patients suspected of having pandemic (H1N1) 2009 infection might have been falsely categorized as non-infected due to late sampling. However, the design of the study is advantageous in its focus on a defined group of admitted patients suspected of having the infection, thus enabling reliable validation of demographic, clinical and laboratory data on admission and during the patients' hospital course, as well as their clinical outcomes. Obviously, it should be emphasized that the results of the study are not applicable to the course of pandemic (H1N1) 2009 virus infection in the non-admitted general population, but rather to patients whose clinical presentation was severe enough to justify their admission. In addition, our study was performed during a limited period at the beginning of the pandemic (H1N1) 2009 virus outbreak in Israel (July–August 2009). Therefore, as the characteristics of the pandemic might have changed during the subsequent months, some of our study's conclusions might not be applicable to the situation in 2010.

In conclusion, the data indicate that young and relatively healthy pandemic (H1N1) 2009 virus-infected patients constitute a risk group for a severe clinical course. However, mortality, at least in the present series, was confined to patients with significant concurrent diseases. Interestingly, the data indicate that lymphopenia, sometimes profound, was significantly more common among H1N1-positive patients with severe disease, in accordance with a previous report [3]. Moreover, as shown here, kinetic analysis of the lymphocyte counts among ICU patients during hospitalization suggested a correlation between the level of lymphopenia and severity of the disease. Furthermore, the data also suggest that the absence of recovery in the lymphocyte counts despite oseltamivir therapy might predict a poor outcome among ICU patients. In accordance, a previous study performed in H5N1-infected mice showed that infection with the lethal strains of the virus correlated with a profound drop in the lymphocyte counts following their apoptosis, accompanied by diminished synthesis of cytokines such as interleukin-1 and interferon-gamma [13]. This suggests that certain pathogenic strains of influenza virus might severely harm the immune system, resulting in disseminated and lethal disease, a mechanism that might explain the present observations. Therefore, we suggest that lymphopenia and its kinetics during hospitalization in severely ill H1N1-infected patients might serve as a surrogate marker for the severity of the disease and its prognosis.

In summary, our study shows that both age < 65 and the presence of cough are independent predictors for pandemic (H1N1) 2009 virus positivity among clinically suspected

patients. Notably, in contrast to the typical seasonal flu that usually complicates patients in the extreme age groups and who have significant comorbidity, the current pandemic has an unexpected tendency to complications in young and previously healthy people. The more severe cases are characterized by increased inflammatory markers, along with significant lymphopenia. As lymphopenia was associated with disease severity and its clinical course, we suggest using the lymphocyte count and its kinetics as a surrogate marker for disease severity and prognosis.

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References

1. Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 2009; 460: 1021-5.
2. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 2009; 459: 931-9.
3. Chowell G, Bertozzi SM, Colchero MA, et al. Severe respiratory disease concurrent with the circulation of H1N1 influenza. *N Engl J Med* 2009; 361: 674-9.
4. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med* 2009; 361: 680-9.
5. Pandemic alert level 6: scientific criteria for an influenza pandemic fulfilled. *Euro Surveill* 2009; 14: 19237.
6. Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. *N Engl J Med* 2009: 361(20): 1935-44.
7. Kumar A, Zarychanski R, Pinto R, et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 2009; 302: 1872-9.
8. Louie JK, Acosta M, Winter K, et al. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA* 2009; 302: 1896-902.
9. Senanayake SN. Swine flu update: bringing home the bacon. *Med J Aust* 2009; 191: 138-40.
10. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373-83.
11. Michaelis M, Doerr H, Cinatl J. Novel swine-origin influenza A virus in humans: another pandemic knocking at the door. *Med Microbiol Immunol* 2009; 198: 175-83.
12. Szyper-Kravitz M. The hemophagocytic syndrome/macrophage activation syndrome: a final common pathway of a cytokine storm. *IMAJ Isr Med Assoc J* 2009; 11: 633-4.
13. Tumpey TM, Lu X, Morken T, Zaki SR, Katz JM. Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A (H5N1) virus isolated from humans. *J Virol* 2000; 74: 6105-16.
14. Monto AS. The risk of seasonal and pandemic influenza: prospects for control. *Clin Infect Dis* 2009; 48(Suppl 1): S20-5.
15. Ikonen N, Strengell M, Kinnunen L, et al. High frequency of cross-reacting antibodies against 2009 pandemic influenza A(H1N1) virus among the elderly in Finland. *Euro Surveill* 2010;15.

Capsule

Gut inflammation provides a respiratory electron acceptor for Salmonella

Salmonella enterica serotype Typhimurium (*S. typhimurium*) causes acute gut inflammation by using its virulence factors to invade the intestinal epithelium and survive in mucosal macrophages. The inflammatory response enhances the transmission success of *S. typhimurium* by promoting its outgrowth in the gut lumen through unknown mechanisms. Winter and collaborators show that reactive oxygen species generated during inflammation react with endogenous, luminal sulphur compounds (thiosulphate) to form a new respiratory electron acceptor, tetrathionate. The genes conferring the ability to use tetrathionate as an electron acceptor produce a growth advantage for *S. typhimurium* over the competing microbiota in the lumen of the inflamed gut. The authors conclude that *S. typhimurium* virulence factors induce host-driven production of a new electron acceptor that allows the pathogen to use respiration to compete with fermenting gut microbes. Thus the ability to trigger intestinal inflammation is crucial for the biology of this diarrhoeal pathogen.

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Eitan Israeli

Capsule

Nets of DNA fibers and deep vein thrombosis

Nets of DNA fibers and antimicrobial proteins in blood vessels ensnare and kill microbes during infection, and may also provide a scaffold for blood clots. The immune system relies on these meshes, known as neutrophil extracellular traps, to fight infection. Wagner of the Immune Disease Institute in Boston, Massachusetts, and her colleagues found that the nets also catch platelets – cell fragments that aid in blood clotting. The nets bound additional proteins known to stabilize clots, and DNA markers of the nets were found in the blood and clots of baboons with deep vein thrombosis – a condition in which blood clots form in deep-seated veins. This finding could explain the link that has been made in humans between this condition and infection.

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Eitan Israeli

Novel Multitargeted Anticancer Oral Therapies: Sunitinib and Sorafenib as a Paradigm

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ABSTRACT: The introduction of novel targeted therapies into the clinic in recent years has had a considerable impact on the management of several neoplastic diseases – such as gastrointestinal stromal tumors, hepatocellular carcinomas and renal cell carcinomas – considered until recently refractory to systemic therapies. We describe here two such novel biological agents, sunitinib and sorafenib, as a paradigm of the successful clinical application of new concepts. Sunitinib and sorafenib are small molecule tyrosine kinase inhibitors that target vascular endothelial growth factor receptor, platelet-derived growth factor receptor, C-Kit and others. Both agents are administered orally; sunitinib is typically given in cycles for 4 consecutive weeks with 2 weeks off, while sorafenib is given continually. Side effects occur in most patients, similar for both agents; they may affect several systems and organs but are mostly mild and easily manageable, rarely requiring discontinuation of the drug. However, these toxicities mandate prompt attention and intervention. The most frequently observed effects are hypertension, nausea, anorexia, asthenia and cutaneous manifestations; cardiac abnormalities may include congestive failure. Sunitinib, and markedly less frequently sorafenib, may cause thyroid gland dysfunction, mainly hypothyroidism. Antitumor activity has been shown for renal cell carcinoma in pivotal trials, for sunitinib as first-line treatment and for sorafenib in previously treated patients as second-line. Sunitinib is now approved as second-line therapy for patients with GIST refractory to imatinib; sorafenib has resulted in a significant prolongation in median survival in patients with hepatocellular carcinoma. Ongoing clinical trials will further define the spectrum of these agents' antitumor activity, their role in combination with other drugs, as well as their optimal dose and schedule of administration.

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KEY WORDS: multitargeted anticancer oral therapies, sunitinib, sorafenib, tyrosine kinase inhibitors, renal cell carcinoma, hepatocellular carcinoma, gastrointestinal stromal tumors

Recent years have witnessed a dramatic expansion of the anticancer drug armamentarium, including both cytotoxic agents and biologicals. For the first time we have active targeted therapies against diseases that until very recently were traditionally considered refractory to systemic manipulations, such as renal cell and hepatocellular carcinomas. The purpose of this review is to update the clinical experience available on two novel multitargeted small molecule tyrosine kinase inhibitors now in routine use – sunitinib and sorafenib.

SUNITINIB

Sunitinib (Sutent®, Pfizer Inc, USA) is a multitargeted tyrosine kinase inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor, the stem cell factor receptor C-Kit, and others. Its main mechanism of action is through inhibition of tumor angiogenesis although it also has antiproliferative and apoptosic effects on diverse tumor types [1]. Sunitinib is metabolized by the cytochrome P450 3A4 system; strong inhibitors of this system such as cimetidine, erythromycin, ketoconazole and others, may increase plasma concentration of the drug, while CYP 3A4 inducers (such as barbiturates and corticosteroids) may decrease its plasmatic levels [2].

Typically, sunitinib is given at a dose of 50 mg once daily for 4 consecutive weeks followed by a 2 week rest. The dose does not need to be adjusted for age, weight, gender or performance status; however, pharmacokinetic data in patients with severe impairment of renal or hepatic functions are still lacking.

Sunitinib may result in a number of side effects that require close monitoring and, frequently, dose adjustments, temporary interruption, and sometimes even discontinuation of the drug. Patients must be informed about the potential side effects; they should learn how to identify them and should be encouraged to report them in real time to the treating physician. One should be aware that side effects of multitargeted drugs have a distinct pattern of toxicities that indeed differ from that traditionally observed with cytotoxic chemotherapeutic agents. The incidence of serious grade 3–4 toxicities is generally low, but they require prompt intervention.

The range of potential adverse effects is wide and may affect many systems and organs including gastrointestinal, cardiovas-

GIST = gastrointestinal stromal tumors

Table 1. Sunitinib: incidence of frequent side effects

	All grades	Grades 3–4
Arterial hypertension	25%	8%
Left ventricular dysfunction	12%	2%
Thyroid function tests abnormalities	85%	2%
Anorexia	29%	4%
Nausea and vomiting	33%	4%
Diarrhea	51%	6%
Stomatitis	30%	2%
Fatigue	52%	8%
Skin discoloration	30%	–
Hand and foot syndrome	20%	5%
Myelosuppression	70%	6%
Bleeding (epistaxis)	18%	< 1%

cular, endocrinologic and metabolic, dermatologic, and others [Table 1]. Arterial hypertension, thyroid gland dysfunction, and cardiac and skin toxicities demand particular attention. Hypertension as a result of the anti-angiogenic effect of sunitinib occurs frequently in up to 25% of the treated population and should be managed with standard antihypertensive drugs, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, some of the calcium channel blockers, etc. [3]. However, antihypertensive drugs known to inhibit the CYP 3A4 systems such as verapamil should be avoided. Initially, blood pressure should be monitored weekly and then at regular intervals.

Sunitinib and sorafenib represent an example of a new class of anticancer agents, namely, multitargeted oral small molecule tyrosine kinase inhibitors, resulting in inhibition of tumor angiogenesis

In severe hypertension, observed in about 8% of all cases, sunitinib needs to be discontinued temporarily until hypertension is controlled. If hypertension cannot be controlled, sunitinib is permanently discontinued, but this is rarely necessary. Interestingly, treatment-related hypertension has been correlated with an increased response rate in patients with metastatic renal cell carcinoma receiving sunitinib [4].

Sunitinib may cause left ventricular dysfunction in about 10% of patients, and is clinically symptomatic in 1–2%. Baseline and periodic determination of left ventricular ejection fraction is necessary during treatment with sunitinib, particularly in patients with known cardiac risk factors. In patients with a decrease in left ventricular ejection fraction of ≤ 20% from baseline or below 50%, sunitinib should be temporarily interrupted; if overt congestive heart failure develops sunitinib should be discontinued [5]. Other potential cardiac events that are rarely observed include bradycardia and QT prolongation; therefore, the concomitant administration of sunitinib with QT prolonging agents such as halopendol, quinolone and macrolide antibiotics, and anti-emetics such as ondansetron and granisetron should be avoided.

Close monitoring of thyroid function is mandatory for patients receiving sunitinib. Thyroid function test abnormalities have been described in up to 85% of patients with renal cell carcinoma undergoing treatment with sunitinib. These abnormalities may occur very early, within 1–2 weeks from the onset of treatment with sunitinib, mostly hypothyroidism with elevated thyroid-stimulating hormone and decreased triiodythyronine and more rarely thyroxine, resulting in complaints such as fatigue, anorexia, fluid retention and intolerance to cold. Thyroid hormone replacement should be instituted promptly in these patients [6]. Rarely, thyrotoxicosis may precede hypothyroidism but burnout occurs rapidly and once hypothyroidism develops it may be profound. In patients on sunitinib, monitoring of thyroid-stimulating hormone should start at baseline and continue every 2–3 months during therapy as the incidence of hypothyroidism increases with more prolonged use of sunitinib. Sunitinib appears to cause follicular cell apoptosis with subsequent thyroiditis [6].

Sunitinib may cause a variety of dermatologic toxicities that develop typically 3 to 4 weeks from the start of treatment. These include yellow skin discoloration, observed in up to one-third of the patients, which is reversible upon discontinuation of the drug; hair depigmentation may occur as well, with normally pigmented hair regrowing during the off-treatment period. Yellow discoloration of the skin is due to the color of sunitinib and its metabolites, and hair depigmentation is caused by inhibition of melanocyte function through blockage of C-Kit signaling [7].

Painful hand and foot syndrome with erythema, edema and blisters on the palms and soles and occasionally with numbness and dysesthesia may occur frequently after 3 to 4 weeks from the start of sunitinib, requiring the use of moisturizers, skin care products, avoidance of pressure on affected areas, etc. [8]. Generalized skin rashes do occur rarely and are usually mild. Subungueal splinter hemorrhages may also be observed and oral changes such as dry mouth and stomatitis, usually mild, may occur.

Gastrointestinal side effects include anorexia in 10–30% of the patients, nausea and vomiting (mostly moderate) and severe in less than 5% of the patients, and diarrhea in about 50% of the patients on sunitinib, being severe in about 5% of cases. In severe cases, sunitinib is discontinued until the diarrhea resolves and then resumed at a lower dose [9].

Profound myelosuppression from sunitinib, through inhibition of C-Kit, is uncommon, with grades 3–4 neutropenia and/or thrombocytopenia reported in less than 10% of patients, with blood counts usually returning to normal

promptly. Blood counts should be obtained at the start of each new cycle; for recurrent grades 3–4 toxicity dose reduction of sunitinib is warranted. Mild bleeding, most commonly epistaxis, has been reported in up to one-fifth of patients on treatment with sunitinib; severe life-threatening bleeding is exceedingly rare [9].

Several laboratory abnormalities have been reported in patients taking sunitinib, including elevated lipase and amylase levels, without overt pancreatitis. Other laboratory abnormalities have included changes in glycemia, calcium, phosphorus and potassium, all of which can be readily corrected [10].

Clinical antitumor activity of sunitinib was initially demonstrated for renal cell carcinoma. The pivotal phase III clinical trial reported initially by Motzer et al. in 2007 [11] compared sunitinib with interferon-alpha as first-line therapy in 750 patients with metastatic clear cell renal cell carcinoma. The response rate was 37% for sunitinib and 9% for interferon, and the median progression free survival was 11 months and 5 months respectively for sunitinib and interferon ($P < 0.001$), establishing the former as standard first-line treatment for patients with metastatic renal cell carcinoma. In a recent update of their work [12], an overall survival advantage for sunitinib was also demonstrated (26.4 months median survival as compared to 21.8 months with interferon, $P = 0.05$). For patients who did not receive further therapies, the difference in median survival was more robust, 28 months for sunitinib vs. 14 months for interferon ($P = 0.0033$) [12].

Sunitinib and sorafenib are active in entities unresponsive to cytotoxic chemotherapy such as renal cell and hepatocellular carcinomas and gastrointestinal stromal tumors

In an international expanded access study of sunitinib in metastatic renal cell carcinoma, 4185 patients representing a heterogeneous population were included; some had been previously treated and a few had a low performance status; nevertheless, the median progression-free survival reached 11 months and the median survival was 19.8 months [13].

Maintaining adequate plasmatic levels of sunitinib seems to be important. A meta-analysis of three trials (two phase II and one phase III) in renal cell carcinoma showed that higher exposure to sunitinib was associated with an increased probability of response and a longer time to progression and survival [14]. It is in this regard that the administration of sunitinib using a continuous daily schedule at a dose of 37.5 mg is being investigated.

Ongoing studies with sunitinib in renal cell carcinoma include its administration in combination with other targeted therapies. Such a strategy is based on the simultaneous blockage of several signal transduction pathways. The administration of sunitinib and bevacizumab (Avastin®, Roche, Switzerland) is being investigated since the activity and safety of this combination has not yet been fully established.

The use of sunitinib as second-line therapy in renal cell carcinoma is also being pursued. In a recently published trial sunitinib was given following progression of the disease with sorafenib; an 18% partial response rate was achieved, and in 55% of the patients the disease stabilized. No correlation was found between response to sorafenib and subsequent benefit with sunitinib, suggesting limited cross-resistance between both agents [15]. Furthermore, the potential impact of sunitinib in the adjuvant setting following resection of the primary kidney tumor is being investigated in several clinical studies, including the STRAC trial (Sunitinib Treatment of Renal Adjuvant Cancer) assessing one year of sunitinib compared to placebo in high risk localized renal cell carcinoma after nephrectomy [16]. The three-arm designed ASSURE trial (Adjuvant Sunitinib and Sorafenib in Unfavorable Renal Cell Carcinoma) compares sunitinib vs. sorafenib vs. placebo following nephrectomy [16]. An EORTC trial is presently investigating the use of sunitinib before (neoadjuvant) or after (adjuvant) nephrectomy [16].

In addition to renal cell carcinoma, sunitinib is active against gastrointestinal stromal tumors. In a phase III clinical trial, 312 patients with GIST who had been treated with imatinib (Glivec®, Novartis, Switzerland) were randomized to receive either sunitinib or placebo. The median time to progression was 27 weeks with sunitinib and 6 weeks in the placebo arm ($P < 0.0001$), with a median overall survival advantage for the sunitinib-treated group (74 vs. 36 weeks, $P < 0.001$) [17]. A worldwide study in patients with GIST who were ineligible for a clinical trial or where clinical trials were not available included 1117 patients who were treated with sunitinib; their median time to progression was 41 weeks and the median survival reached 75 weeks. Prognostic factors affecting outcome included age, performance status and prior dose of imatinib [18]. Due to the potential risk for disease progression during the 2 week break of sunitinib administration, a trial of sunitinib given daily at a dose of 37.5 mg is ongoing in patients with GIST who progressed on imatinib. Of note, current data indicate that median survival times on treatment with sunitinib in patients with GIST are longer for C-Kit exon 9 mutations or wild-type c-kit/PDGFR as compared to C-Kit exon 11 mutations, contrary to experience with imatinib where best responses are observed in patients with C-Kit exon 11 tumor mutations [19]. The concomitant administration of sunitinib and imatinib is now under investigation in patients with GIST whose disease progressed after imatinib alone.

PDGFR = platelet-derived growth factor receptor

SORAFENIB

Sorafenib (Nexavar®, Bayer Pharmaceuticals, USA) is a novel oral multitargeted thyrosine kinase inhibitor of VEGFR, PDGFR, C-Kit and the RAF-1 protein. It induces both tumor apoptosis and disruption of the tumor vasculature.

Metabolism of sorafenib occurs primarily in the liver by the CYP 3A4 system and by glucuronidation mediated by UGTIA9. The concomitant use of CYP 3A4 inducers may result in reduced plasma levels of sorafenib, but its metabolism is apparently not influenced by CYP 3A4 inhibitors. The co-administration of sorafenib with cytotoxic drugs that are conjugated by UGTIA1, such as irinotecan, docetaxel and doxorubicin, can result in increased levels of these agents [20]. Sorafenib is given at a dose of 400 mg twice daily, without dose adjustments for age, gender, weight or renal function. The dose of sorafenib for patients with pronounced liver dysfunction has not yet been established.

Side effects from sorafenib are similar to some, but not all, of those described in the previous section for sunitinib, and include fatigue, diarrhea, nausea, hand and foot syndrome, alopecia, bleeding and arterial hypertension.

Sunitinib and sorafenib have a wide spectrum of potential side effects clearly different from those typically observed with cytotoxic chemotherapy, including arterial hypertension, thyroid gland dysfunction, cardiac and skin toxicities and metabolic disturbances, all of which require close monitoring

Thyroid dysfunction is much less common than observed with sunitinib. Evaluation of thyroid function was carried out in 39 patients with renal cell carcinoma receiving sorafenib. While 21% had abnormalities in thyroid function tests, only 5% developed clinical manifestations of hypothyroidism [21]. Temporary dose reduction to 400 mg/day or the same dose every 2 days and/or discontinuation may be necessary for the management of side effects.

Sorafenib exerts antitumor activity in renal cell carcinoma as well. A phase III TARGET clinical trial by Escudier et al. [22] compared sorafenib with placebo in previously treated metastatic clear cell carcinoma of the kidney. The median progression-free survival was significantly longer in the sorafenib-treated patients, 24 weeks vs. 12 weeks in the placebo arm ($P < 0.001$); when an interim analysis was undertaken, 80% of the patients in the sorafenib group remained progression-free. These results led to Food and Drug Administration approval of sorafenib as second-line treatment in metastatic renal cell carcinoma.

Results of the TARGET trial were recently updated; overall survival analysis censoring the placebo patients demonstrated a survival advantage for sorafenib: 17.8 vs. 14.3 months, respectively [23].

VEGFR = vascular endothelial growth factor receptor

Ongoing studies with sorafenib include its use as first-line therapy in renal cell carcinoma, alone compared to interferon, or in combination with the latter. Progression-free survival was similar for sorafenib and interferon as single agents at 6 months, but the sorafenib-treated patients had higher rates of tumor shrinkage and better quality of life scores. Clinical benefit was observed among patients in whom the dose of sorafenib was increased to 600 mg twice a day or with sorafenib crossover following progression of the disease on interferon [24]. The concomitant use of sorafenib and sunitinib in renal cell carcinoma is currently under investigation.

Sorafenib has been given in combination with bevacizumab in patients with renal cell carcinoma; while the response rate was substantial, 46% of 48 patients, increased toxicity was observed, mainly hand-foot syndrome and hypertension [25].

Sorafenib has also shown antitumor activity in collecting duct carcinoma, a rare variant of renal cell carcinoma [26], and in papillary and chromophobe renal cell carcinoma [27]. The use of sorafenib in the adjuvant setting in renal cell carcinoma is being investigated in the ASSURE trial (Adjuvant Sorafenib and Sunitinib in Unfavorable Renal Cell Carcinoma) [16]. The SORCE trial is comparing 1 or 3 years of adjuvant sorafenib compared to placebo following surgery in patients with primary renal cell carcinoma with intermediate or high risk for relapse [16].

Sorafenib is active in hepatocellular carcinoma, typically a chemoresistant disease entity. In a landmark phase III multicenter clinical trial, 602 patients with advanced hepatocellular carcinoma previously unexposed to systemic therapy were randomized to either placebo or sorafenib. Patients in the sorafenib arm experienced a significantly longer median survival, 10.7 vs. 7.9 months ($P < 0.001$) and median time to radiologic progression (5.5 vs. 2.8 months, $P < 0.0012$), when compared to placebo, a gain of about 3 months, notwithstanding a low objective remission rate of only 2% [28]. It should be emphasized that antitumor response rates to single-agent biologicals are consistently low when applying traditional Response Evaluation Criteria in Solid Tumors (RECIST), with a scarcity of complete remissions. The degree of tumor necrosis as evaluated by imaging studies rather than changes in overall tumor size seems to correlate better with patient outcome, prompting the adoption of novel, more appropriate endpoints to evaluate response to these agents. The Choi criteria, for instance, define a partial response as a decrease in tumor size of only 10% (or more), but with a decrease in tumor density of > 15% on computed tomography [29]. In patients with GIST following progression of the disease after both imatinib and

sunitinib, sorafenib resulted in a median survival of 13 months with 62% of the patients being alive at 1 year [30].

CONCLUDING REMARKS

In summary, the recent ongoing development of targeted therapies is having a substantial impact on the management of several malignant diseases. These agents have resulted in major achievements that influence the clinical course and prognosis of entities that until now were traditionally considered unresponsive to cytotoxic therapies, including renal cell carcinoma, hepatocellular carcinomas and GIST. Further directions include investigations on the optimal use of these newer agents in sequence or in combination, and on their place in the adjuvant and neoadjuvant settings.

Sequential administration enables maintaining a continuum of treatment with agents each given at full dose, targeting different pathways at different times. Sunitinib and sorafenib serve as a paradigm of the clinical impact achieved with a new generation of agents recently incorporated in to the anticancer armamentarium. Some targeted therapies are scheduled to continue for prolonged periods; the prompt and early detection of side effects is of utmost importance to reduce patient discomfort and to avoid dose reductions and/or interruptions of treatment.

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References

1. Mendel DB, Laird AD, Sin X, et al. In vivo antitumor activity of SU-11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: Determination of a pharmacokinetic-pharmacodynamic relationship. *Clin Cancer Res* 2003; 9: 327-37.
2. Lacy CF, Armstrong LL, Goldman MP. Cytochrome P-450 enzymes and drug metabolism In: Lacy DV, Armstrong LL, Goldman MP, eds. Drug Information Handbook. 12th edn. Hudson, OH, 2004: 1619-31.
3. Sico DA. Angiogenesis inhibitors and hypertension: an emerging issue. *J Clin Oncol* 2006; 24: 1329-31.
4. Rixe O, Billemont B, Izzedine H. Hypertension as a predictive factor of sunitinib activity. *Ann Oncol* 2007; 18: 1117.
5. Chu TF, Repnick MA, Kerkela R, et al. Cardiotoxicity associated with tyrosine kinase inhibitor Sunitinib. *Lancet* 2007; 370: 2001-19.
6. Rini BI, Tamaskar I, Shaheen P, et al. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. *J Natl Cancer Inst* 2007; 99: 81-3.
7. Moss KG, Toner GC, Cherrington JM, et al. Hair depigmentation is a biological readout for pharmacological inhibition of KIT in mice and humans. *J Pharmacol Exp Ther* 2003; 307: 476-80.
8. Robert C, Soria JC, Spatz A, et al. Cutaneous side effects of kinase inhibitors and blocking antibodies. *Lancet Oncol* 2005; 6: 491-500.
9. Motzer RJ, Rini BI, Bukowski RM. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006; 295: 2516-24.
10. Kollmannsberger C, Soulieres D, Wong R, et al. Sunitinib therapy for metastatic renal cell carcinoma: recommendations for management of side effects. *Can Urol Assoc J* 2007; 1: S41-54.
11. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; 356: 115-24.
12. Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; 27: 3584-90.
13. Sternberg CN. Expanding the boundaries of clinical practice: building on experience with targeted therapies. *Ann Oncol* 2009; 20(Suppl 1): il-6.
14. Houk B, Bello LL, Michaelson MD, et al. A population/pharmacodynamic analysis of exposure-response for sunitinib in metastatic renal cell carcinoma. *Eur J Cancer* 2007; 5(Suppl): 299.
15. Zimmerman K, Schmittel A, Steiner U, et al. Sunitinib treatment for patients with advanced clear-cell renal-cell carcinoma after progression on sorafenib. *Oncology* 2009; 76: 350-4.
16. Bellmunt J. Future developments in renal cell carcinoma. *Ann Oncol* 2009; 20(Suppl 1): i13-17.
17. Demetri GD, Van Oosterom AT, Garrett R, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumors after failure of imatinib: a randomized controlled trial. *Lancet* 2006; 368: 1329-38.
18. Reichardt P, Kang Y, Ruka W, et al. Detailed analysis of survival and safety with sunitinib in a worldwide treatment-use trial in patients with advanced GIST. *J Clin Oncol* 2008; 26 (May 20 suppl): Abstr 10548.
19. Heinrich MC, Maki RG, Corless CL, et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib maleate in imatinib-resistant gastrointestinal stromal tumors. *J Clin Oncol* 2008; 26: 5352-9.
20. Hutson TE, Figlin RA, Kuhn JG, et al. Targeted therapies for metastatic renal cell carcinoma: an overview of toxicity and dosing strategies. *Oncologist* 2008; 13: 1084-96.
21. Tamaskar I, Bukowski R, Elson P, et al. Thyroid function test abnormalities in patients with metastatic renal cell carcinoma treated with sorafenib. *Ann Oncol* 2008; 19: 265-8.
22. Escudier B, Eisen T, Stadler WM. Sorafenib in advanced clear-cell renal cell carcinoma. *N Engl J Med* 2007; 356: 125-34.
23. Bukowski RM, Eisen T, Szczylik C, et al. Final results of the randomized phase III trial of sorafenib in advanced renal cell carcinoma: survival and biomarker analysis. *J Clin Oncol* 2007; 25(June 20 suppl): Abstr 5023.
24. Szczylik C, Demkow T, Staehler M, et al. Randomized phase II trial of first line treatment with sorafenib versus Interferon in patients with advanced renal cell carcinoma. Final results. *J Clin Oncol* 2007; 25: 5025-50.
25. Azad NS, Posadas EM, Kwitkowski VE, at al. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. *J Clin Oncol* 2008; 26: 3709-14.
26. Ansari J, Fatima A, Chaudhri S, et al. Sorafenib induces therapeutic response in a patient with metastatic collecting duct carcinoma of kidney. *Onkologie* 2009; 32: 44-6.
27. Chouriri TK, Plantade A, Elson P, et al. Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. *J Clin Oncol* 2008; 26: 127-31.
28. Llovet JM, Ricci S Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; 359: 378-90.
29. Choi H, Charnsangavej C, Faria SC, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. *J Clin Oncol* 2007; 25: 1753-9.
30. Nimeiri HS, Maki RG, Kasaza K, et al. Activity of sorafenib in patients with Imatinib and sunitinib-resistant gastrointestinal stromal tumors (GIST): a phase II trial of the University of Chicago phase II Consortium. Soc Clin Oncol GI Cancers Symposium, 2008: Abstr 7m.

The Proper Place for the Committer of a Crime is Prison Custody not Psychiatric Hospital Inpatient Care

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KEY WORDS: closed forensic unit, prison custody, district psychiatric board

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The issue of involuntary hospitalization in Israel is highly controversial: it is a meeting point between the medical world and the Law. With much interest I read the article by Bergman-Levy and co-authors on the British experience, appearing in this issue of the journal [1]. True, the care and treatment of violent patients is consuming a larger and larger portion of staff time. True also, that the courts are referring increasingly more defendants to the psychiatric system for diagnosis and treatment. Yet, I am not persuaded that Britain's experience is relevant to us in Israel or that we should copy their model. The size of the two populations is very different; the population mix is different, and no less different is the legislation governing the two care and treatment systems. This is not the place, I think, to set out the details of these disparities, the more so as we have yet to say a word on the economic aspect. Is the British model really worth the large financial investment required to replicate it here? This is not at all evident.

I agree that the current situation in Israel regarding this issue is not good, as I will point out later. The single psychiatric hospital in Israel with a maximum security classification is Sha'ar Menashe Mental Health Center's forensic unit. This unit's four wards and 120 beds are supposed to meet the needs of all Israel's psychiatric hospitals as well as of its own local community. It is obvious that the care and treatment of dangerous patients require special resources and a great deal of time, so it is no surprise that the Sha'ar Menashe unit gets rapidly blocked up with too many patients and no vacant beds and so becomes incapable of meeting the dynamically changing needs of the population of dangerous patients. To this must of course be added the logistic and financial hardships that the families of Sha'ar Menashe patients have to bear in order to visit the remotely located facility. The evident solution is to construct a closed forensic unit in each of Israel's six regions, *but not in every mental health center*. In my opinion these units could be either freestanding or part of an existing closed ward, provided they are designed to be escape-proof. The semantic issue of their designated security level is immaterial as long as the unit is escape-proof. The truly "difficult" patients, however, are the ones that such units will not usually be able to handle, and they will still need to be sent to the Sha'ar Menashe maximum security unit.

But the above model is certainly not the only possible solution and it is the purpose of this editorial to propose an alternative and totally different method based on ideas quite different to those driving Israel's existing forensic psychiatry care system. The core idea is that all court-ordered compulsory hospitalizations be transferred whole and without change from our mental health centers to the Israel Prison Service's custodial facilities and that patients be treated there, just as they are currently treated in the Magen Asher prison's mental health care wards in Ramle, in central Israel. The guiding principle here is that the proper place for someone who has committed a crime – even if the act is the outcome of mental illness – is prison custody and not psychiatric hospital inpatient care. I am guided by the premise that the public interest outweighs the interest of a mentally ill defendant, so that even when it is clear that he committed his act because of the illness afflicting him and in some cases is not fit to stand trial, he should nonetheless be transferred to a custodial facility equipped to provide him with all the necessary medical care in a specialist mental health care unit. After treatment and stabilization of his mental state he will then proceed, like any Israel Prison Service inmate, along the institutional track to conditional release. That is, the local District Psychiatric Board for his facility will decide, just as it currently does, whether he can be given furlough, long or short, with or without escort, or full release, or that he needs to remain in full psychiatric custodial inpatient care.

The second phase of the patient's care, shortly before his final release from custodial hospitalization, will be the issuing of a Compulsory Outpatient Care Order, which will instruct the patient/inmate to attend regular treatment sessions in his prison's outpatient clinic. This treatment will include the periodic monitoring of his state of mental health. That the treatment will take place in a prison clinic gives us the vital advantage that, should the patient/inmate not persist with his treatment or not appear for a checkup at the designated times, he can then be returned to full psychiatric custodial inpatient care, just as at present a prisoner 'on licence' who violates the conditions of

his 'time off for good behavior' is returned to prison to serve out the full term of his court-ruled sentence.

At this point, the question has to be asked how long can someone be held in full psychiatric custodial inpatient care only for violating the release conditions laid down by the District Psychiatric Board, when it converted his Compulsory Hospitalization Order (CHO) to a Compulsory Outpatient Care Order (COCO). The answer I offer is that the overall time should be the same as the length of the prison sentence the patient/inmate would have got for his offense had he not been found to be mentally ill.

I am fully convinced that the change in the systemic approach proposed here will not only solve the problem of our current 'toothlessness' in enforcing Compulsory Outpatient Care Orders, it will also prevent a relapse into the psychotic state for which the patient/inmate was hospitalized in the first place. I also expect that a further favorable side effect will be fewer applications to courts by defense lawyers for psychiatric examination orders, the alleging of insanity being their customary first line of defense on behalf of their suspect/detainee client. This practice of defense lawyers puts an unnecessary and unjustifiable burden on both the courts and the psychiatric care system. For this reason alone – to stop the cheapening of psychiatric examinations – I have been campaigning for years for the proposal put forward here, but unfortunately with no success.

I hope that my response to the article by Bergman-Levy et al. will, at the least, provoke a productive discussion of the issue in question. A sharp sidelight is thrown on it by the Haifa District Court's [2] ruling a few months ago that the proviso of insanity does not apply when the psychotic state obtaining when an accused person committed his criminal act stemmed from the abuse of, or withdrawal from, drugs. For the insanity proviso to apply, the court has ruled, it must be shown that it was by reason of mental illness not by reason of drug addiction that the accused lacked the capacity to stop himself committing the act in question. Before this ruling, accused persons who, when they committed an offense were in a drug abuse-induced psychotic state, were sent to a mental health center under Article 15 of the Mental Patients Treatment Act. Was there any justification for this practice? Of course not. Such offenders should be sent to an Israel Prison Service custodial facility and have their psychotic state treated there. I am gratified that the courts have now reached the same conclusion and I only hope that this ruling will lead the psychiatric treatment system to change its stance on the issue discussed here.

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References

1. Bergman-Levy T, Bleich A, Kotler M, Melamed Y. Security and psychiatry: the British experience and implications for forensic psychiatry services in Israel. *IMAJ Isr Med Assoc J* 2010; 12: 587-91.
2. Haifa District Court Criminal Appeals 6235-07-09

Capsule

European population substructure correlates with systemic lupus erythematosus endophenotypes in North Americans of European descent

Previous work has demonstrated that Northern and Southern European ancestries are associated with specific systemic lupus erythematosus (SLE) manifestations. In a study by Richman et al., 1855 SLE cases of European descent were genotyped for 4965 single-nucleotide polymorphisms, and principal components analysis of genotype information was used to define population substructure. The first principal component (PC1) distinguished Northern from Southern European ancestry, PC2 differentiated Eastern from Western European ancestry and PC3 delineated Ashkenazi Jewish ancestry. Compared with Northern European ancestry, Southern European ancestry was associated with autoantibody production [odds ratio (OR) 1.40, 95% confidence interval (CI) 1.07–1.83] and renal involvement (OR 1.41, 95% CI 1.06–1.87), and was protective for discoid rash (OR 0.51, 95% CI 0.32–0.82) and photosensitivity (OR 0.74, 95% CI 0.56–0.97). Both serositis (OR 1.46, 95% CI 1.12–1.89) and autoantibody production (OR 1.38, 95% CI 1.06–1.80) were associated with Western compared to Eastern European ancestry. Ashkenazi Jewish ancestry was protective against neurologic manifestations of SLE (OR 0.62, 95% CI 0.40–0.94). Homogeneous clusters of cases defined by multiple PCs demonstrated stronger phenotypic associations. Genetic ancestry may contribute to the development of SLE endophenotypes and should be accounted for in genetic studies of disease characteristics.

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Eitan Israeli

"Advice is seldom welcome, and those who need it the most, like it the least"

Lord Chesterfield (1896-1973), British far right-wing politician and journalist who helped found right-wing organizations in Britain, primarily in opposition to the break-up of the British Empire, and later adopted a broader anti-immigration stance.

Infections More than Vaccines are Inducers of Autoimmune Diseases

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The evolvement of immune responses against self-antigens and the development of autoimmune diseases are multifactorial in origin. The most important of these factors are the genetic susceptibility of the individual and the environmental factor, mainly viruses and bacteria. Molecular mimicry and increased expression of modified, cryptic, or new antigenic determinants are among the many antigen-specific mechanisms. Nonspecific mechanisms known as bystander activation include enhanced processing, presentation of self-antigens, immune cell activation, cytokine release, and cell apoptosis [1].

INFECTIONS AND AUTOIMMUNE DISEASES

The correlation between cytomegalovirus and Raynaud's phenomenon in lupus nephritis was demonstrated in early studies. In this regard, serologic analyses for CMV, parvovirus B19 and Epstein-Barr virus were performed in 60 patients with systemic lupus erythematosus and evaluated for the presence of vascular events, Raynaud's phenomenon and antiphospholipid syndrome. It was observed that CMV seropositivity was a highly significant risk factor for Raynaud's phenomenon but not for venous vascular events [2]. The molecular mimicry of the EBV peptide PPPGRRP by the peptide PPPGMRPP from SmB'/B of the human nuclear antigen strengthened the possibility that in some cases EBV infection is associated with the onset of SLE. With this in mind, James et al. [3] tested SLE patients and matched controls for evidence of previous infection with EBV. All but one of the196 patients with SLE had been exposed to EBV, whereas 22 of 392 healthy controls had no previous EBV exposure (odds ratio 9.35, 95% confidence interval 1.45–infinity, $P = 0.014$). These findings strongly supported the role of EBV in the development of SLE.

In order to identify environmental agents that could potentially induce autoimmunity, autoantibodies against 60 kDa Ro in SLE were found to cross-react with a peptide from the latent viral protein EBV nuclear antigen-1. Animals immunized with each of these antigens progressively developed autoanibodies binding multiple epitopes of Ro autoantigens, but they also developed clinical symptoms of lupus such as leukopenia, thrombocytopenia and renal failure. This further supports the notion that autoimmune responses in human SLE arise through molecular mimicry between viral antigens and autoantigens [4].

Assessing the impact of acute viral infections on the diagnosis and management of 88 SLE patients, Ramos-Casals and co-authors [5] noted that 25 patients were diagnosed with new-onset SLE associated with infection by human parvovirus B19 (n=15), CMV (n = 6), EBV (n = 3), and hepatitis A virus (n = 1). The remaining 63 cases of acute viral infections arose in patients already diagnosed with SLE. The most common viral infections in patients with SLE were parvovirus B19 (predominantly mimicking SLE presentation) and CMV infection, which was shown to mimic a lupus flare or present with specific organ involvement such as pulmonary infiltrates [5].

The role of viral infections in the induction of other autoimmune diseases has also been explored. Early epidemiologic studies suggested an association between EBV infection and risk of multiple sclerosis. Antibody titers to EBV viral capsid antigen, nuclear antigens (EBNA-1, and EBNA-2) and diffuse and restricted early antigen (EA-D and EA-R) as well as to CMV were analyzed. Of 62,439 women who gave blood samples, definite or probable MS was found in 144, while 288 age-matched women were defined as normal. Compared with healthy controls, women with MS had higher serum geometric mean titers of antibodies to EBV but not to CMV. The strongest association was found for antibodies to EBNA-2; and a fourfold difference in titers was associated with a 3.9 relative risk of MS (95%CI 1.1–13.7). These results strongly support a role for EBV in the etiology of MS [6]. In a later study, blood specimens for the detection of anti-EBV were collected up to 30 years prior to the onset of MS. Titers of antibodies to EBNA-1 were significantly higher in the MS cases when compared with matched controls, suggesting again that elevation of anti-EBV titers is probably an early event in the pathogenesis of

CMV = cytomegalovirus

EBV = Epstein-Barr virus
SLE = systemic lupus erythematosus

EBNA = EBV nuclear antigen-1
MS = multiple sclerosis
CI = confidence interval

MS [7]. In line with this is the proposal that vaccination against EBV may prevent MS, and that effective antiviral drugs will inhibit disease progression in patients with MS and may even be curative [8].

Many microbial antigens were also shown to induce cross-reactive immune responses against self-antigens and enhance their presentation to the immune system. For example, about a third of all cases of Guillain-Barré syndrome were preceded by *Campylobacter jejuni* infection, which expresses a lipopolysaccharide molecule that mimics gangliosides that were found to be increased in peripheral nerves [9]. These observations raised the debate regarding whether autoimmune diseases could also possibly be triggered by vaccines.

VACCINATION AND AUTOIMMUNE DISEASES

Most vaccines "work" better when adjuvants are added to them. Adjuvants, from the Latin word "adjuvare" meaning "to help," are compounds used to enhance a vaccine's ability to elicit a strong, durable and protective immune response. They make vaccines more effective by inducing higher T cell activity and a better B cell memory with higher neutralizing and long-lasting antibodies. Of all the adjuvants, the use of Toll-like receptor agonists (in many vaccines, e.g., for human papillomavirus, hepatitis B and influenza A) greatly improves the vaccines' efficacy and established the era of modern, efficient and safe vaccination [10].

The medical literature is replete with case reports exemplifying the risk of autoimmune diseases as a possible consequence of vaccination. Whereas autoimmune diseases occur in 5% of individuals in developed countries, vaccination-related autoimmunity remains very rare and in most reported cases lacks firm confirmation [11-13]. During the last 100 years, billions of people received a variety of vaccines, many of which are adjuvanted with different compounds (aluminum salts, oils, Toll-like receptors). In the vast majority of all vaccine recipients, there were no side effects. Therefore, the issue of linking autoimmune diseases with vaccination remains highly questionable.

SURVEILLANCE STUDIES FOR SAFETY AFTER VACCINATION

The Vaccine Adverse Event Reporting System was established in the United States in 1990 to register spontaneously reported vaccination-induced adverse events. This repository comprises the largest cohorts from which one can learn about the incidence of adverse events following any vaccination. According to these reports, GBS after influenza vaccinations in persons 18 years or older were evaluated for each influenza season from 1990 through 2003. During this period VAERS received 501 reports of GBS following influenza vaccination in adults. The annual reporting rate decreased fourfold from a high of 0.17 per 100,000 vaccinees in 1993–1994 to 0.04 in 2002–2003 ($P < 0.001$) [14]. In a VAERS report from 2003 summarizing adverse events from 1991 through 2001, the overall dose-based reporting rate for the 27 frequently reported vaccine types was 11.4 reports per 100,000 net doses distributed. The most commonly reported adverse event was fever, which appeared in 25.8% of all reports, followed by injection site hypersensitivity (15.8%), rash (unspecified) (11.0%), injection site edema (10.8%), and vasodilation (10.8%). Of all the reports 14.2% described serious adverse events, which by regulatory definition include death, life-threatening illness, hospitalization, or permanent disability. Reviews of VAERS reports during this period demonstrated that vaccines are usually safe and that serious adverse events do occur but are extremely rare [15]. Later, in a report from the U.S. Centers for Disease Control / Food and Drug Administration / VAERS, analyzing 54 reports of GBS following vaccination against influenza, hepatitis and other diseases, it was concluded that vaccines other than influenza could be associated with GBS [16]. Contrary to the above, other studies showed no significant increase in the development of GBS, and the risk of its development after vaccination was judged to be substantially lower than the risk for severe influenza and influenza-related complications [12].

It appears that the association between hepatitis B infection and the development of MS does not occur in individuals following vaccination. This possibility was first noted in France following a report of 35 cases of primary demyelinating events occurring between 1991 and 1997 and within 8 weeks of recombinant hepatitis B vaccine injection [17]. Of importance is that definite MS was diagnosed in only half the patients after a mean follow-up of 3 years. Nearly 25 million people (40% of the population of France) received the hepatitis B vaccine during this period, of whom 18 million were adults. At least ten studies did not find a possible significant association between hepatitis B vaccination and the occurrence of MS in any of these vaccinees. In support of these results, findings of another two large-scale studies showed no significant association between hepatitis B vaccination and the development of MS [18].

There are numerous reports in the literature demonstrating a connection between vaccination and the development of immune mediated inflammation or autoimmunity. A transiently or persistently increased level of autoantibodies (antinuclear, cardiolipin, or extractable nuclear antigen antibodies) was demonstrated in up to 15% of apparently healthy adults after influenza vaccination [19]. However, the development of autoimmune diseases such as autoimmune thrombocytopenia, or SLE-like phenomena, antiphospholipid syndrome and rheumatoid arthritis is quite rare. Some researchers, though rarely, have claimed that post-vaccination morbidity may appear even 10 years after vaccination [20].

The possible development of antiphospholipid syndrome 6 months after a diph-

GBS = Guillain-Barré syndrome
VAERS = Vaccine Adverse Event Reporting Systems

theria-tetanus vaccination is discussed by Meyer and co-authors in this issue of *IMAJ*, who suggest a potential connection between this vaccination and the appearance of this autoimmune disease [21].

The question whether vaccination is safe in patients with rheumatic diseases such as rheumatoid arthritis or SLE is continuously raised. Addressing this question, many studies proved vaccines (against influenza or *Streptococcus pneumoniae*) to be safe in these diseases, especially in remission [22-24]. It is crucial that the incidence of vaccine-related disorders be compared with that associated with the corresponding natural infection for the whole population. Clearly, vaccine-related autoimmunity is possible and we should be aware of it. Furthermore, appropriate prospective and multicenter epidemiologic studies should be designed. Both clinical and laboratory data should be included in the process of long-term post-vaccination monitoring. At this stage it is our duty to encourage the wide use of vaccination, especially when indicated, but to keep our eyes open and be aware of possible vaccine-related adverse events.

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References

1. Sfriso P, Ghirardello A, Botsios C, et al. Infections and autoimmunity: the multifaceted relationship. *J Leukoc Biol* 2010; 87: 385-95.
2. Stratta P, Canavese C, Ciccone G, et al. Correlation between cytomegalovirus infection and Raynaud's phenomenon in lupus nephritis. *Nephron* 199; 82: 145-54.
3. James JA, Neas BR, Moser KL, et al. Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum* 2001; 44: 1122-6.
4. McClain MT, Heinlen LD, Dennis GJ, Roebuck J, Hareley JB, James JA. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat Med* 2005; 11: 85-9.
5. Ramos-Casals M, Cuadrado MJ, Alba P, et al. Acute viral infections in patients with systemic erythematosus: description of 23 cases and review of the literature. *Medicine (Baltimore)* 2008; 87: 311-18.
6. Ascherio A, Munger KL, Lennette ET, et al. Epstein-Barr Virus antibodies and risk of multiple sclerosis: a prospective study. *JAMA* 2001; 286: 3083-8.
7. DeLorenze GN, Munger KL, Lennette ET, Orentreich N, Vogelman JH, Ascherio A. Epstein-Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. *Arch Neurol* 2006; 63: 839-44.
8. Pender MP. Preventing and curing multiple sclerosis by controlling Epstein-Barr virus infection. *Autoimmun Rev* 2009; 8: 563-8.
9. Wraith DC, Goldman M, Lambert PH. Vaccination and autoimmune disease: what is the evidence? *Lancet* 2003; 362: 1659-66.
10. Agmon-Levin N, Paz Z, Israeli E, Shoenfeld Y. Vaccination and autoimmunity. *Nat Rev Rheumatol* 2009; 5: 648-52.
11. Vial T, Descotes J. Autoimmune diseases and vaccination. *Eur J Dermatol* 2004; 14: 86-90.
12. Chen RT, Pless R, Destrefano F. Epidemiology of autoimmune reactions induced by vaccination. *J Autoimmun* 2001; 16: 309-18.
13. Agmon-Levin N, Shoenfeld Y. Chronic fatigue syndrome with autoantibodies – the result of an augmented adjuvant effect of hepatitis-B vaccine and silicone implant. *Autoimmun Rev* 2008; 8: 52-5.
14. Haber P, DeStefano F, Angulo FJ, et al. Guillain-Barre syndrome following influenza vaccination. *JAMA* 2004; 292: 2478-81.
15. Zhou W, Pool V, Iskander JK, et al. Surveillance for safety after immunization: Vaccine Adverse Event Reporting System (VAERS) – United States, 1991-2001. *MMWR Surveill Sum* 2003; 52: 1-24.
16. Souayah N, Nasar A, Suri MF, Qureshi AI. Guillain-Barre syndrome after vaccination in the United States: a report from CDC/FDA Vaccine Adverse Event Reporting System. *Vaccine* 2007; 25: 5253-5.
17. Tourbah A, Gout O, Liblau R, et al. Encephalitis after hepatitis B vaccination: recurrent disseminated encephalitis or MS? *Neurology* 1999; 53: 396-401.
18. Ascherio A, Zhang SM, Hernan MA, et al. Hepatitis B vaccination and the risk for multiple sclerosis. *N Engl J Med* 2001; 344: 327-32.
19. Toplak N, Kveder T, Trampus-Bakija A, Subelj V, Cucnik S, Avcin T. Autoimune response following annual influenza vaccination in 92 apparently healthy adults. *Autoimmun Rev* 2008; 8: 134-8.
20. Ryan AM, Bermingham N, Harrington HJ, Keohane C. Atypical presentation of macrophagic myofasciitis 10 years post vaccination. *Neuromuscul Disord* 2006; 16: 867-9.
21. Meyer A, Rotman-Pikielny P, Natour A, Levy Y. Antiphospholipid syndrome following a diphtheria-tetanus vaccination: coincidence versus causality. *IMAJ Isr Med Assoc J* 2010; 12: 638-9.
22. Holvast B, Huckriede A, Kallenberg CG, BiJl M. Influenza vaccination in systemic lupus erythematosus: safe and protective? *Autoimmun Rev* 2007; 6: 300-5.
23. Elkayam O, Ablin J, Caspi D. Safety and efficacy of vaccination against streptococcus pneumonia in patients with rheumatic diseases. *Autoimmun Rev* 2007; 6: 312-14.
24. Conti F, Rezai S, Valesini G. Vaccination and autoimmune rheumatic diseases. *Autoimmun Rev* 2008; 8: 124-8.

Capsule

Prevalence and burden of pediatric-onset systemic lupus erythematosus

Reviewing the prevalence and burden of pediatric-onset systemic lupus erythematosus (pSLE), Kamphuis & Silverman found that pSLE represents 10–20% of all SLE cases, and is associated with higher disease severity, including more-rapid damage accrual, than adult-onset SLE. Owing to improvements in disease management and recognition over the past 20–30 years, patients now live longer, but as a result have increased disease damage. Premature atherosclerosis and osteoporosis have become increasingly prevalent morbidities in pSLE patients. Early atherosclerosis leads to a considerable rise in cardiovascular and cerebrovascular events, and failure to develop adequate peak bone mass during adolescence – a crucial period of bone accrual – is likely to lead to early osteoporosis and fractures. Patients with pSLE have an incurable, potentially devastating disease that occurs during a vulnerable period of psychosocial development, leading to specific and unique psychosocial stressors.

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Eitan Israeli

"Every person has a history and a geography"

R.S. Downie, British writer

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Antiphospholipid Syndrome Following a Diphtheria-Tetanus Vaccination: Coincidence vs. Causality

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KEY WORDS: antiphospholipid, tetanus, vaccine, autoimmune diseases

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For Editorial see page 635

The association between autoimmune disease and vaccines has been proposed in numerous reports in the past. We present here a case of a healthy young male who received a diphtheria-tetanus vaccination and was diagnosed with antiphospholipid syndrome a few months later.

PATIENT DESCRIPTION

The patient was a previously healthy 28 year old student. Six months before his admission to our ward, he received a diphtheria-tetanus toxoid vaccination because of a laceration. Two weeks prior to the current admission, he was referred to the emergency department because of pleuritic chest pain. His blood tests and chest X-ray were normal and he was discharged. He returned to the ED 2 days later with a fever of 38.2ºC. A chest X-ray revealed an infiltration in the right lower lobe. The patient was given amoxicillin/clavulanate and discharged. Three days later, he returned to our ED with complaints of hemoptysis, cough and persistent fever. Chest X-ray showed an infiltration on the right lower lobe, with a small amount of pleural effusion and atelectasis. He was admitted to the pulmonary department. Antibiotic treatment was changed to moxifloxacin after consultation with the infectious diseases department. The patient's condition improved under this treatment and he was discharged after 5 days of therapy, afebrile with amelioration of his respiratory symptoms. Two days after discharge he began to experience leg pain that worsened gradually, followed by swelling of the left calf. Five days after the last discharge he returned to the ED, this time with a suspected deep vein thrombosis. Doppler ultrasonography was performed, demonstrating a thrombus in the popliteal and saphenous veins. He was admitted to our ward, and subcutaneous injections of enoxaparin and oral warfarin were given.

The patient had no history of prior venous or arterial thromboembolism and denied smoking. There was no known family history of thromboembolism. He denied weight loss, night sweats, pruritus, or change in bowel habits. A chest computed tomography was performed, which revealed a pulmonary embolism that probably explains the earlier clinical picture of non-resolving pneumonia.

We performed a workup for thrombophilic states, which showed a high lupus anticoagulant titer and low activated protein C resistance. Three months after discharge, a second test for lupus anticoagulant was positive and the diagnosis of antiphospholipid syndrome was made. The patient was also heterozygous to factor 5 Leiden.

ED = emergency department

COMMENT

Numerous reports have discussed a possible association between vaccines and autoimmune diseases. One of the suggested mechanisms is the "molecular mimicry" theory, according to which an antigen of the vaccine resembles a host antigen, leading to an autoimmune process. Another theory is that immunization may cause the appearance of, or an increase in immune complexes, which may induce vasculitis or exacerbate a preexisting autoimmune disease [1]. Patients with a genetic predisposition for an autoimmune disease may be at higher risk for developing one after a vaccination. Nevertheless, most epidemiologic studies have not found a direct causal link between vaccinations and the onset of an autoimmune disease [2].

Autoantibodies to various phospholipids have been identified in patients with antiphospholipid syndrome, resulting in a wide spectrum of clinical phenomena such as arterial and venous thrombosis, microvascular thrombosis, placental insufficiency, and a variety of other manifestations. Despite the well-established clinical picture, certain pathogenetic issues remain unanswered, especially regarding the initial triggering events leading to a thrombosis.

There is little information on the connection between immunization and antiphospholipid antibodies. In one study, 85 healthy students were vaccinated with a recombinant hepatitis B virus vaccine. One month later, a minor, statistically insignificant rise in antiphospholipid antibodies was detected [3]. Recently, Inic-Kanada et al. [4] showed that mice

monoclonal antibodies against diphtheria-tetanus toxoid cross-react with β2 glycoprotein and cause adverse pregnancy outcomes when injected in BALB/c mice. These outcomes can mimic the pathogenesis of antiphospholipid syndrome in humans.

Agmon-Levin et al. [5] recently proposed that each component of the vaccine can induce autoimmunity via several mechanisms. Yet, large epidemiologic studies have failed to demonstrate post-vaccination autoimmunity.

Possible causal relationships between vaccinations and autoimmunity have long been discussed, but have not been proven. We present here a patient with clinically and serologically proven antiphospholipid syndrome that manifested a few months after a diphtheria-tetanus vaccine. A possible pathogenic role of the vaccine in the appearance of antiphospholipid syndrome cannot be excluded.

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References

1. Agmon-Levin N, Kivity S, Shoenfeld Y. Influenza vaccine and autoimmunity. *IMAJ Isr Med Assoc J* 2009; 11: 183-5.
2. Molina V, Shoenfeld Y. Infection, vaccines and other environmental triggers of autoimmunity. *Autoimmunity* 2005; 38: 235-45.
3. Martinuc Porobic J, Avcin T, Bozic B, et al. Antiphospholipid antibodies following vaccination with recombinant hepatitis B vaccine. *Clin Exp Immunol* 2005; 142: 377-80.
4. Inic-Kanada A, Stojanovic M, Zivkovic I, et al. Murine monoclonal antibody 26 raised against tetanus toxoid cross-reacts with β2-glycoprotein 1: its characteristics and role in molecular mimicry. *Am J Reprod Immunol* 2009; 61: 39-51.
5. Agmon-Levin N, Paz Z, Israeli E, Shoenfeld Y. Vaccines and autoimmunity. *Nat Rev Rheumatol* 2009; 5: 648-52.

Capsule

Origin of the human malaria parasite *Plasmodium falciparum* in gorillas

Plasmodium falciparum is the most prevalent and lethal of the malaria parasites infecting humans, yet the origin and evolutionary history of this important pathogen remain controversial. Liu et al. have developed a single-genome amplification strategy to identify and characterize *Plasmodium* spp. DNA sequences in fecal samples from wild-living apes. Among nearly 3000 specimens collected from field sites throughout central Africa, the authors found Plasmodium infection in chimpanzees (*Pan troglodytes*) and western gorillas (*Gorilla gorilla*), but not in eastern gorillas (*Gorilla beringei*) or bonobos (*Pan paniscus*). Ape plasmodial infections were highly prevalent, widely distributed and almost always made up of mixed parasite species. Analysis of more than 1100 mitochondrial, apicoplast and nuclear gene sequences from chimpanzees and gorillas revealed that 99% grouped within one of six host-specific lineages representing distinct Plasmodium species within the subgenus Laverania. One of these from western gorillas comprised parasites that were nearly identical to *P. falciparum*. In phylogenetic analyses of full-length mitochondrial sequences, human *P. falciparum* formed a monophyletic lineage within the gorilla parasite radiation. These findings indicate that *P. falciparum* is of gorilla origin and not of chimpanzee, bonobo or ancient human origin.

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Eitan Israeli

Capsule

Macrophages need food to fight infection

Starvation or poor nutrition weakens the immune system and makes organisms more susceptible to disease. This reflects in part the need for adequate energy stores. But Mieulet and team provide evidence that sufficient uptake of the amino acid arginine is required for mouse macrophages to respond to bacterial lipopolysaccharide, which binds to Toll-like receptors that initiate innate immune responses. Macrophages deprived of arginine appeared to have a deficit in the activation of mitogen-activated protein kinases (MAPKs) that mediate the immune response. In cultured cells deprived of arginine, the protein kinase TPL-2 (tumor-promoting locus 2, a MAPK kinase kinase) was associated to a greater extent with protein phosphatase 2A, leading to dephosphorylation and inactivation of TPL-2. In mice, deprivation of arginine also reduced MAPK activation and the consequent production of tumor necrosis factor-alpha. Arginine thus appears to have multiple important roles in the innate immune response: It serves as a substrate for the synthesis of nitric oxide as part of cellular response to bacterial infection, and it maintains a key signaling pathway that allows macrophages to fight infection effectively.

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Eitan Israeli

"How easy to be amiable in the midst of happiness and success"

Madame Anne Sophie Swetchine (1782-1857), Russian mystic

Acute Pancreatitis may be Caused by H1N1 Influenza A Virus Infection

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KEY WORDS: pancreatitis, H1N1 influenza, viral infection

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Influenza A (H1N1) virus is a subtype of influenza virus A and the most common cause of influenza in humans. Some strains of H1N1 are endemic in humans and cause a small fraction of all influenza-like illness and a large fraction of all seasonal influenza. H1N1 strains caused roughly half of all human flu infections in 2006 [1]. Other strains of H1N1 are endemic in pigs (swine influenza) and birds (avian influenza). In June 2009, the World Health Organization declared that flu due to a new strain of swine-origin H1N1 was responsible for the 2009 flu pandemic. This strain is often called "swine flu" by the public media.

Influenza A virus strains are categorized according to two proteins found on the surface of the virus: hemagglutinin (H) and neuraminidase (N). All influenza A viruses contain hemagglutinin and neuraminidase, but the structures of these proteins differ from strain to strain due to rapid genetic mutation in the viral genome. Influenza A virus strains are assigned an H number and an N number based on which forms of these two proteins the strain contains. There are 16 H and 9 N subtypes known in birds, but only H 1, 2 and 3, and N 1 and 2 are commonly found in humans [2]. In 2009 more than 99% of circulating influenza viruses identified in the United States were 2009 H1N1 influenza, previously referred to as novel influenza A (H1N1). The clinical presentation of patients with uncomplicated 2009 H1N1 influenza virus infection is generally similar to seasonal influenza and includes abrupt onset of fever, cough, sore throat, myalgias, arthralgias, chills, headache and fatigue. Vomiting and diarrhea have been reported more often with 2009 H1N1 flu than with seasonal flu. As with seasonal flu, some patients with 2009 H1N1 flu present without fever.

The U.S. Centers for Disease Control recommends early empiric antiviral treatment for suspected or confirmed influenza in hospitalized patients and in outpatients at higher risk for complications (http://www.cdc.gov/h1n1flu/recommendations.htm). Empiric antiviral treatment, when indicated, should be initiated as early as possible and should not be delayed pending the results of influenza testing.

PATIENT DESCRIPTION

We report a 37 year old man who was admitted with epigastric abdominal pain that started on the day of admission. Three days earlier he started to suffer high fever (38°C) together with sore throat, myalgia and malaise.

On the day of admission he had severe epigastric abdominal pain without nausea or vomiting. The physical examination was normal except for tender upper abdomen, but without rigidity. Blood pressure was 95/54 mmHg, 80 beats per minute, 96% O_2 saturation, 12 respirations/min. There were no signs of distress except for the painful abdomen. The cardiorespiratory system was normal, as was the neurologic examination.

Sedimentation rate was 30 mm in the first hour, hemoglobin 13.7 g/dl, 4680 leukocytes/mm^3, and 161,000 platelets/mm^3. Biochemical results were normal except for amylase 600 U/L (that decreased to 70 u/L after 48 hours) with aspartate aminotransferase that was elevated (79 u/L) and returned to normal values (33 u/L) within 24 hours. The international normalized ratio and partial thromboplastin time were normal and the lipid profile was also within normal limits (cholesterol 111 mg/dl, triglycerides 63 mg/dl).

Chest and abdominal computed tomography did not demonstrate any abnormality, the pancreas showed no signs of edema or inflammation, and the gallbladder and the biliary ducts had no stones or sludge and no signs of inflammation.

Since the suspected clinical diagnosis was acute pancreatitis, the patient was treated with a combination of antibiotics and Tamiflu® (Roche) 75 mg twice daily after a throat culture for H1N1 influenza virus swabs was taken from the throat and the nostrils and found to be positive for H1N1.

Twenty-four hours from the start of the combined treatment with antibiotics and Tamiflu, the patient's condition improved dramatically and most of his symptoms disappeared.

COMMENT

Viruses can cause acute pancreatitis, but this is the first description of a possible H1N1 influenza A infection causing acute pancreatitis that resolved quite quickly. Our patient may represent a new clinical presentation of H1V1 Influenza A infection – the "swine flu" type. Although he did not have all the requisite signs of acute

pancreatitis, he had upper abdominal pain with high levels of amylase that returned to normal within 24 hours. The abdominal CT did not demonstrate signs of pancreatitis or biliary tract disease; however, the abdominal pain with the high amylase level could suggest acute pancreatitis. His low sedimentation rate and low leukocyte count may also suggest a viral infection that affected the pancreas.

Several reports have described viral pancreatitis: acute pancreatitis caused by mumps in a 34 year old woman [1], patients with human immunodeficiency virus [2,3], and patients infected with hepatitis E [4] and hepatitis A [5]. It was found that pancreatitis remains a significant cause of morbidity in the HIV population in the HAART era.

Acute pancreatitis is associated with female gender, severe immunosuppression, and use of stavudine and aerosolized pentamidine [3]. The risk of acute pancreatitis among patients with HIV has been significantly associated with age, race, symptomatic HIV infection, and liver and cardiovascular disease [3]. One of the patients described suffered acute pancreatitis while he had an acute infection (immunoglobulin M antibodies) with hepatitis E, and the common etiologies were excluded [5]. Another reported patient had acute pancreatitis caused by hepatitis A [5].

Our patient had acute upper abdominal pain together with high fever and symptoms and signs of an acute viral infection; this was diagnosed as an acute infection with the H1N1 influenza A virus, the "swine flu" type. To the best of our knowledge this is the first report of this clinical condition, which may suggest that H1N1 virus can cause acute pancreatitis.

HIV = human immunodeficiency virus
HAART = highly active antiretroviral therapy

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References

1. Taii A, Sakagami J, Mitsufuji S, Kataoka K. Acute pancreatitis from mumps re-infection in adulthood. A case report. *JOP* 2008; 9(3): 322-6.
2. Riedel DJ, Gebo KA, Moore RD, Lucas GM. A ten-year analysis of the incidence and risk factors for acute pancreatitis requiring hospitalization in an urban clinical cohort. *AIDS Patient Case STDS* 2008; 22(2): 113-21.
3. Guo JJ, Jang R, Louder A, Cluxton RJ. Acute pancreatitis associated with different combination therapies in patients infected with human immunodeficiency virus. *Pharmacotherapy* 2005; 25(8): 1044-54.
4. Makharia GK, Garg PK, Tandon RK. Acute pancreatitis associated with acute hepatitis E infection. *Trop Gastroenterol* 2003; 24(4): 200-1.
5. Khanna S, Vij JC. Severe acute pancreatitis due to hepatitis A virus infection in a patient of acute viral hepatitis. *Trop Gastroenterol* 2003; 24(1): 25-6.

Capsule

Methicillin resistance mechanism in Staphylococcus

Almost since methicillin was first introduced as an antibiotic in the 1960s, resistant bacteria were detected, but no one has been quite sure where this form of defense originated. Resistance is encoded by *mec* on a transmissible cassette chromosome that spreads among staphylococcal bacteria, including the sometime hospital resident, *Staphylococcus aureus*. Tsubakishita et al. investigated, by genetic means, the wild relatives of *S. aureus*. They found in *S. fleurettii* (a commensal bacterium of domesticated animals) the original chromosomal locus that served as the template for the cassette; it appears that the cassette was formed by the combination of *mec* with the mobile element. *mec* genes comprise four classes, with *mecA* in *S. fleurettii* being the prototype and sharing almost complete nucleotide identity with *mecA* of methicillin-resistant *S. aureus*. Two scenarios are posited: that new resistant cassettes are continuously generated in staphylococci or that animal commensals act as a reservoir for human resistance.

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Eitan Israeli

Capsule

Self-renewing T cells

The homeostasis of cell populations within an organism can be achieved through a variety of mechanisms, including the differentiation of precursor populations, self-renewal of terminally differentiated cells, or by programming cells to be extremely long lived. Regulatory T cells that express the transcription factor Foxp3 are critical for maintaining immune tolerance by preventing excessive inflammation and autoimmunity. Rubtsov and researchers used genetic fate mapping and cell transfer studies in vivo to demonstrate that Foxp3-expressing cells are remarkably stable under both basal and inflammatory conditions. Thus, regulatory T cells appear to be maintained through self-renewal and should maintain their identity if used in adoptive cell therapies for treatment of autoimmunity or other inflammatory disorders.

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Colon and Lung Choriocarcinoma

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KEY WORDS: choriocarcinoma of colon, lung choriocarcinoma, non-gestational choriocarcinoma

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Choriocarcinoma is a rare tumor that usually arises in the uterus and gonads. However, it can also occur in extragenital locations such as the lung [1], mediastinum [2], retroperitoneum [3] and gastrointestinal tract [4]. Choriocarcinoma of the gastrointestinal tract is extremely rare. It is usually located in the stomach, esophagus, small bowel and colon. Only nine cases of colon and rectal choriocarcinoma have been reported in the English-language medical literature. In addition, choriocarcinoma of the colon and the lung in association with adenocarcinoma of the contralateral lung has never been reported. We present the first case of a coexisting colon and lung choriocarcinoma with lung adenocarcinoma, describe the management, and review the literature on this unique clinical presentation.

PATIENT DESCRIPTION

A 57 year old woman with a history of heavy smoking presented with hemoptysis. Chest X-ray showed bilateral shadowing in the upper lobes. Chest computed tomography scan demonstrated bilateral infiltrative lesions. Fine needle aspiration revealed non-small cell carcinoma in both lungs. Positron emission tomography-CT demonstrated uptake in the lung lesions and in retrocaval and paratracheal lymph nodes (stage IV disease). Before undergoing a combined chemotherapy and immunotherapy protocol the patient was required to undergo a full-body CT, which demonstrated a tumor in the left colon. Colonoscopy revealed a left colon lesion and biopsy showed poorly differentiated adenocarcinoma. At this time it was assumed that the patient had two primary lesions: adenocarcinoma of the lung and adenocarcinoma of the colon. Although the patient was asymptomatic we decided to perform a left colectomy to prevent colonic obstruction or bleeding during chemotherapy. At surgery, a polypoid tumor in the splenic flexure was detected. Left colectomy was performed, the postoperative course was uneventful and the patient was discharged after 8 days.

The pathologic finding was a solid malignant tumor invading the muscularis propria and composed of two populations of cells: large polygonal clear cells and atypical multinucleated giant cells. Giant cells were strongly positive for human chorionic gonadotropin and negative for carcinoembryonic antigen. Eight negative lymph nodes were resected. Histology and the immunohistochemical pattern of the tumor were consistent with choriocarcinoma.

An immunohistochemical dye showed that the colon biopsy that had been taken during colonoscopy demonstrated choriocarcinoma rather than adenocarcinoma as previously assumed.

Further history taking revealed that the patient had had an abortion. This raised the possibility of a microgerminal cell tumor from a hydatiform mole, but transvaginal ultrasound was interpreted as normal. Serum levels of βhCG were 13,000 IU/L before chemotherapy. The patient received four courses of VIP combination chemotherapy (VP-16 100 mg/m^2, ifosfamide 1.2 g/m^2, cisplatin 20 mg/m^2) and mesna 1.2 g/m^2. The chemotherapy cycles were one every 3 weeks, with each cycle given for 4 days. βhCG levels decreased by a log per course to less than 5 IU/L after three courses. A PET-CT scan later detected only the two lesions in the lungs. Eight weeks later a right upper lung lobectomy revealed adenocarcinoma. After recovery a left upper lobectomy was performed, and the histologic appearance showed interstitial fibrosis surrounded by foam macrophages and necrotic tissue, which is expected following chemotherapy. However, immunohistochemical staining showed one area that was strongly positive for βhCG. The patient did not receive further chemotherapy and died 16 months after the diagnosis of choriocarcinoma was made, with metastasis to the bone and brain.

COMMENT

Choriocarcinoma is a germ cell tumor that can be gestational or non-gestational. Non-gestational choriocarcinoma is a rare malignancy that can occur in the lung [1], mediastinum, kidney, stomach, small bowel and large bowel [2]. Gestational choriocarcinoma usually occurs in young females and has no connection with gonadal choriocarcinoma, which is more common in males. Both gestational and non-gestational choriocarcinoma are associated with a high serum level of βhCG.

There have been nine cases of colonic

βhCG = beta-human chorionic gonadotropin

PET-CT = positron-emission tomography-CT

choriocarcinoma reported in the English literature [Table]. Of these, all but two were associated with adenocarcinoma. In this report, the two remote lesions, both in the colon and the lungs, showed a combination of two different histologic characteristics as described above.

This patient was totally asymptomatic with regard to the colon choriocarcinoma, in contrast to previous reports of gastrointestinal choriocarcinoma. The PET-CT detected only the lung lesion, emphasizing the problematic issue of PET-CT as a low sensitivity diagnostic modality in choriocarcinoma. Knowing the patient had had an abortion raises the possibility of microgerminal cell tumor from a hydatiform mole. However, the normal report of transvaginal ultrasound made this option unlikely.

It was speculated by McKechnie and Fechner [4] that choriocarcinoma may originate from adenocarcinoma. Even if no histologic evidence of adenocarcinoma is documented, this theory could still be valid if adenocarcinoma was ruled out by choriocarcinoma [5]. The histopathologic report in our case found no indication for adenocarcinoma in the colon. In this complex case, the source of the tumor is conjectural since choriocarcinoma rarely arises also in the lung. The diagnosis of choriocarcinoma in the colon was suspected by the histologic hematoxylin and eosin examinations and was confirmed by high βhCG serum levels and immunohistochemistry. The patient received four courses of VIP, and serum βhCG levels returned to normal towards the end of the chemotherapy regimen. When first measured, 3 weeks after the colectomy, βhCG levels were 13,000 IU/L. βhCG decreased to 27 IU/L after the first and second courses of chemotherapy and only after the third course reached normal levels (< 5I U/L). The half-life of βhCG is 24 hours and this raised concern that the choriocarcinoma was active.

Since time is critical following the diagnosis of choriocarcinoma, prompt treatment with chemotherapy is crucial for prolonging survival if there is metastatic spread. In the present case the reduction of βhCG in the serum indicates response to treatment. The choriocarcinoma responded to chemotherapy only after three courses of VIP. βhCG did not

VIP = VIP-16 100 mg/m², ifosfamide 1.2 g/m², cisplatin 20 mg/m²

Reported cases of colon choriocarcinoma

Author, journal (yr)	Patient's age (yrs) and gender	Tumor site	Management	Outcome
Park et al. *Cancer* (1980)	49 female	Sigmoid chorio- and adenocarcinoma with metastasis	Hartman's operation + 5 FU	Patient died 1 month post-surgery from liver and cardiopulmonary insufficiency
Gia-Khanh Nguyen *Dis Col Rectum* (1982)	74 male	Sigmoid chorio- and adenocarcinoma	Hartman's operation	Patient died 10 weeks after bowel resection with liver metastasis. Elevated levels of βhCG were documented
Hitoshi Kubosawa et al. *Cancer* (1984)	50 female	Sigmoid chorio- and adenocarcinoma	Hartman's operation	Patient died 5.5 weeks after bowel resection with liver, lung, parapancreatic lymph node metastasis
Ordonez & Luna *Am J Gastroenterol* (1984)	35 female	Right colon chorio- and adenocarcinoma	Right colectomy	Patient died 10 weeks after bowel resection with metastasis to liver, lungs, pleura, pericardium, iliac bone, mediastinal, mesenteric and periaortic lymph nodes
Lind et al. *Am J Clin Pathol* (1986)	42 male	Metastatic right colon choriocarcinoma	Laparotomy, whole brain irradiation and chemotherapy with bleomycin and cisplatin	Patient died 1 month following admission. Metastases were found in both lungs, paratracheal lymph nodes, liver, spleen, kidneys and paraaortic lymph nodes. High probability for bone and brain metastases as well
Mitsuru Tokisue et al. *J Gastroenterol* (1996)	29 female	Rectal chorio- and adenocarcinoma	Chemotherapy: 4 courses of methotrexate, etoposide, actinomycin-D. Tumor resection, another two courses of methotrexate, etoposide, actinomycin-D, cisplatin, doxorubicin	Patient died 10 months after treatment initiation. Although both primary (rectal) foci and pulmonary metastasis showed regression after chemotherapy, pulmonary metastasis enlarged and brain metastasis were detected
Kiran et al. *Eur J Surg Oncol* (2001)	68 male	Distal colon chorio- and adenocarcinoma. 31 month after Hartman's operation for rectal carcinoma	Tumor resection	Patient died of liver failure before chemotherapy was begun
Duy T. Le et al. *Dis Colon Rectum* (2003)	73 male	Metastatic Rt colon choriocarcinoma	Rectal biopsy	Patient died 10 days after admission. Postmortem report revealed a choriocarcinoma in the colon, brain, lungs, pancreas, kidney and in mesenteric lymph nodes
Verbeek et al. *Hum Pathol* (2004)	54 female	Low rectal choriocarcinoma	Abdominoperineal resection, hysterectomy, adnexectomy, paraaortic and locoregional lymph node resection. Chemotherapy 4 cycles of cisplatin, etoposide, ifosfamide. thoracotomy for resection of lung metastasis.	Patient died 8 month after diagnosis

decrease to normal levels after surgery, implying that micrometastatic disease not recognized by PET-CT was still present after surgery.

Choriocarcinoma is a systemic disease and it is sometimes difficult to determine the site of the primary lesion. As choriocarcinoma of the colon and digestive system is rare and usually not identified until the tumor has spread, our assumption is that this patient had a primary colon choriocarcinoma that had spread to her lungs and later to the bones and brain. New tests in the future might be more sensitive for the detection of choriocarcinoma cells [5]. However, the prognosis is worsened by the presence of metastatic disease involving the central nervous system, liver, or gastrointestinal tract.

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References

1. Pushchak MJ, Farhi DC. Primary choriocarcinoma of the lung. *Arch Pathol Lab Med* 1987; 111(5): 477-9.
2. Le DT, Austin RC, Payne SN, Dworkin MJ, Chappell ME. Choriocarcinoma of the colon: report of a case and review of the literature [Review]. *Dis Colon Rectum* 2003; 46(2): 264-6.
3. Ordonez NG, Luna MA. Choriocarcinoma of the colon. *Am J Gastroenterol* 1984; 79(1): 39-42.
4. McKechnie LC, Fechner RE. Choriocarcinoma and adenocarcinoma of the esophagus with gonadotropin secretion. *Cancer* 1971; 27(3): 694-702.
5. Peleg D, Peleg A, Shalev E. Immunodetection of living trophoblast. *IMAJ Isr Med Assoc J* 2000; 2: 821-2.

Capsule

Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma

Severe asthma is associated with the production of interleukin 17A (IL-17A). The exact role of IL-17A in severe asthma and the factors that drive its production are unknown. Lajoie et al. demonstrate that IL-17A mediated severe airway hyperresponsiveness (AHR) in susceptible strains of mice by enhancing IL-13-driven responses. Mechanistically, the authors demonstrate that IL-17A and AHR were regulated by allergen-driven production of anaphylatoxins, as mouse strains deficient in complement factor 5 (C5) or the complement receptor C5aR mounted robust IL-17A responses, whereas mice deficient in C3aR had fewer IL-17-producing helper T cells (T_H17 cells) and less AHR after allergen challenge. The opposing effects of C3a and C5a were mediated through their reciprocal regulation of IL-23 production. These data demonstrate a critical role for complement-mediated regulation of the IL-23–T_H17 axis in severe asthma.

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Eitan Israeli

Capsule

Peripheral innervation is necessary for organogenesis and may be involved in organ repair or regeneration

Organ development requires the differentiation and coordination of nerves and blood vessels with multiple cell types. The peripheral parasympathetic nervous system innervates many organs during embryogenesis; however, the function of this interaction during organogenesis is unclear. By exploiting the close association during development of the parasympathetic ganglion with the mouse embryonic salivary gland epithelium, Knox et al. found that neuronal innervation preserves an epithelial progenitor cell population via muscarinic receptor and epidermal growth factor receptor signaling. These progenitor cells are then maintained in the adult salivary gland. A similar system was observed in the developing prostate gland. Peripheral innervation is thus necessary for organogenesis and may also be involved in organ repair or regeneration.

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Eitan Israeli

"I detest racialism, because I regard it as a barbaric thing, whether it comes from a black man or a white man"

Nelson Mandela (b. 1918), first South African president to be elected in a fully representative democratic election. Before his presidency, Mandela was an anti-apartheid activist, and leader of the African National Congress (ANC). Mandela served 27 years in prison, mostly on Robben Island. Following his release in 1990, he represented his party in the negotiations that led to multiracial democracy in 1994. As president from 1994 to 1999, he frequently gave priority to reconciliation. In South Africa, Mandela is often known as *Madiba*, an honorary title adopted by elders of Mandela's clan. Mandela has received more than 250 awards over four decades, including the 1993 Nobel Peace Prize.

These research projects, undertaken in partial fulfillment of the requirements for the MD degree at Sackler Faculty of Medicine, Tel Aviv University in 2009–2010, were considered the most outstanding of the graduating class

Representational neglect: comparison between the visual and tactile modalities

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Background: Unilateral spatial neglect (USN), a frequent sequel of stroke affecting the right cerebral hemisphere, is a compound neurological syndrome which is characterized by decreased efficiency in the processing of information arriving from the contralesional hemispace (most frequently the left hemispace). The Representational theory of Neglect (RN), introduced by Bisiach and Luzzatti in 1978 [13], claims that the basic fault in USN is a failure to create an exact representation of the outer world; thus, not only do patients fail to respond to left-sided stimuli but they also fail to recover left-sided details of spatial memory – e.g., a well-known place, such as the town square (the "piazza effect").

Objectives: To compare the characteristics of USN for spatially distributed perceived visual information and spatially reconstructed visual information presented serially in the midsagittal plane, and to compare the latter with spatially reconstructed tactile information.

Methods: Eleven right-hemisphere damaged stroke patients with left-side neglect and 8 healthy controls were presented with pairs of two-dimensional geometric shapes for same/different judgment in three testing conditions: a) 'visual static' (VS) – each object exposed in its entirety; b) 'visual dynamic' (VD) – objects moved horizontally (leftward/rightward) behind a central narrow slit exposing only part of the object at one time; and c) 'tactile dynamic' (TD) – a novel method where the blindfolded subject palpates the upper contour of objects similar in appearance to the visual objects, in both rightward and leftward directions, with the index finger of the healthy right hand. In tasks b and c the spatial representation has to be reconstructed mentally from partial, non-lateralized, sensory information. In order to quantify the spatial disturbance, we used a calculated laterality quotient to evaluate the difference between the correct performance on each side relative to the average level of performance ([R-L]*100/[R+L]).

Results: All patients demonstrated left-side disadvantage in at least two of the three tasks, six patients in all three tasks. The average calculated laterality quotients for correct performance was 54.4 for the VS task, and 14.4 and 20.1 for VD and TD tasks, respectively. Detection of left-side differences in VD and TD tasks improved when the left side of the object was presented last (recency effect).

Conclusions: Our novel method for the assessment of representational neglect is able to demonstrate clear contralesional disadvantage in both visual and tactile modalities. Patients who showed representational disturbance in one modality usually showed that also in the other modality, but dissociations occurred. Similar performance rates for the VS and TD tasks (for both groups) indicate the reliability of our novel method. The temporal order of the mental reconstruction process is important; the recency effect, though it does not withdraw from the presence of RN, which is prominent.

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Characterizing microRNA signature as a prognostic factor in diffuse large B cell lymphoma

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Background: Diffuse large B cell lymphoma (DLBCL) is a hematologic malignancy characterized by an acute manifestation with a rapid deterioration of the patient's condition if no treatment is given during the early stages of the disease. MicroRNA molecules are known to be involved in the development of different malignancies. Specific microRNA signatures are known to be related to the prognosis of patients with some of these malignancies

Objectives: To determine whether microRNA signature profiling can distinguish between DLBCL patients with a good prognosis and those with a bad prognosis

Methods: Two groups were defined: good prognosis group (complete remission without relapse of the disease in 5 years) – 40 patients, and bad prognosis group (lack of complete remission or relapse of the disease within 9 months of diagnosis) – 43 patients. We extracted microRNA from the biopsies obtained during diagnosis and compared the microRNA profiles of the two groups by using Rosetta Genomics microRNA chips.

Results: Our findings clearly show that combining hsa-miR-342-3p with hsa-miR-17 produced the best sensitivity (79%) and

specificity (73%) to predict the patient's prognosis, regardless of the disease stage or other clinical parameters.

Conclusions: It may be possible to predict the prognosis of patients with DLBCL, and perhaps change the treatment of patients according to the genetics of the tumor.

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Erythropoietin and cyclophosphamide combination treatment additively enhances immunoglobulin production in mice

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Background: Erythropoietin (EPO) is an important component in the treatment of cancer-related anemia, and it is usually combined with chemotherapy. Cyclophosphamide (CP) is a known cytotoxic alkylating agent used in cancer chemotherapy. The antineoplastic activity of CP at low doses is attributed to enhancement of cellular and humoral immunity. We have previously shown that EPO displays anti-neoplastic activity and that EPO treatment is associated with enhancement of both the humoral and cellular immune responses.

Objectives: To explore the humoral immunomodulatory effects of combining EPO and low dose CP, thus simulating clinical conditions.

Methods: We compared anti-dinitrophenyl (DNP) immunoglobulin (Ig) serum levels in DNP- keyhole limpet hemocyanin (KLH) injected C57BL mice that were treated with either EPO or CP, separately, or with EPO + CP combination. Diluent injection served as a control for CP and EPO treatment. The levels of IgG1, IgG2a and total Ig were measured using enzyme-linked immunosorbent assay in sera samples taken before, and 2 weeks after antigen injection.

Results: CP treatment alone resulted in increased anti-DNP IgG1 serum levels, 2 weeks following antigen administration. In contrast, EPO treatment alone significantly enhanced anti-DNP IgG2a levels. The combined treatment of EPO and CP increased both IgG1 and IgG2a, maintaining the effects of each treatment alone. While neither CP nor EPO alone significantly increased anti-DNP total Ig levels, the combined treatment additively led to their elevation.

Conclusions: Our findings emphasize a potential role for EPO as an immunomodulator, particularly when administered as a part of a combined treatment with CP.

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Capsule

Loss of heterozygosity on chromosome 17q in ichthyosis with confetti (IWC) patients accounts for normal skin patches

Mitotic recombination can cause a cell carrying heterozygous mutations in a tumor suppressor gene to lose the wild-type copy of the gene, setting the cell on the pathway to uncontrolled growth. But can mitotic recombination have beneficial effects in other settings – that is, lead to phenotypic correction of a diseased cell by facilitating loss of the disease-causing mutation? Choate et al. find evidence for this type of event in a rare skin disease called ichthyosis with confetti (IWC). Patients with IWC display severe scaling of the skin but have widespread patches of normal skin that reflect clonal expansion of revertant cells. The revertant cells showed loss of heterozygosity on chromosome 17q and, as a result of mitotic recombination, these cells selectively lost dominant disease-causing mutations in the keratin 10 gene (*KRT10*), but retained the wild-type copy of the gene.

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Eitan Israeli

Capsule

Antimalarial drug candidate

Spiroindolones were discovered as promising antimalarial drug candidates through a high throughput screening approach that should be applicable to a range of neglected infectious diseases. Rottmann et al. present the preclinical profile for an optimized spiroindolone drug candidate, NITD609. They obtained evidence for a decrease in drug sensitivity in strains of the malaria parasite *Plasmodium falciparum* bearing amino acid mutations in the P-type ATPase, indicating possible mechanisms of action and/or resistance.

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