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Program

All sessions take place in the Grand Ballroom, 3rd Floor

SATURDAY, AUGUST 5, 2000

I. Recognition and Screening – In this session experts will review current "state of the art" techniques used to screen for and confirm the presence of selected liver, pancreatic, intestinal or nutritional disorders.

Session Chairs: Edward J. Hoffenberg, MD, Richard Schreiber, MD

0830 - 0840	Introduction *William F. Balistreri, MD*
0840 - 0900	Basic principles of screening *Jane Brock, MD, MSPH*
0900 - 0920	Screening for celiac disease *Edward J. Hoffenberg, MD*
0920 - 0940	Screening for inflammatory bowel disease *Ernest Seidman, MD, FRCP*
0940 - 1000	Questions
1000 - 1030	Break
1030 - 1050	Screening for cystic fibrosis/hereditary pancreatitis *Peter R. Durie, MD*
1050 - 1110	Screening for hemochromatosis and Wilson Disease *Bruce Bacon, MD*
1110 - 1130	Screening for viral hepatitis-pediatric epidemiology *Maureen Jonas, MD*
1130 - 1150	Questions
1150 - 1330	Lunch on your own

II. Mechanism of GI Disease: Insight Into Management – In this session new developments which have led to a greater understanding of certain disorders will be reviewed.

Session Chairs: M. Susan Moyer, MD, Hilary Perr, MD

1330 - 1400	Inborn errors of fatty acid metabolism and liver disease *Piero Rinaldo, MD*
1400 - 1410	Questions
1410 - 1440	The enteric nervous system and GI motility *Carlo DiLorenzo, MD*
1440 - 1450	Questions
1450 - 1520	Healing gastrointestinal mucosa: two sides to the problem *Daniel Podolsky, MD*
1520 - 1530	Questions
1530 - 1550	Break
1550 - 1620	Obesity and the Regulation of body weight and appetite *Lee Kaplan, MD, PhD*
1620 - 1630	Questions
1630 - 1700	Immunobiology of the gastrointestinal tract: Food induced hypersensitivities *Hugh Sampson, MD*
1700 - 1710	Questions

SUNDAY, AUGUST 6, 2000

III. Pediatric Gastroenterology Therapy: Entering the Next Millennium – Speakers in this session will discuss therapeutic strategies for specific disorders, including a unique view of "emerging options" such as gene therapy and CAM (Complementary and Alternative Medicines).

Session Chairs: Linda Book, MD, Joel Rosh, MD

0830 - 0900	Hepatitis prevention and treatment *Mei-Hwei Chang, MD*
0900 - 0930	A look to the future? Gene therapy for gastrointestinal and hepatic diseases *Lynn Jorde, PhD*
0930 - 0955	IBD therapy in the new millennium *James Markowitz, MD*
0955 - 1010	Questions
1010 - 1030	Break
1030 - 1055	Portal hypertension: New Approaches *Deirdre A. Kelly, MD*
1055 - 1120	Improving results of intestinal transplantation *Samuel Kocoshis, MD*
1120 - 1150	Use of complementary and alternative medicine (CAM) in pediatric gastroenterology and hepatology *Doris Strader, MD*
1150	Questions
1150 - 1200	Closing comments

"A Global View of Pediatric Gastroenterology"

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The discussion, views, and recommendations as to medical procedures, choice of drugs and drug dosages herein are the sole responsibility of the authors. Because of rapid advances in the medical sciences, the Society cautions that independent verification should be made of diagnosis and drug dosages. The reader is solely responsible for the conduct of any suggested test or procedure.

Continuing Medical Education

The North American Society for Pediatric Gastroenterology and Nutrition is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians. The NASPGN designates the Postgraduate Course for a maximum of 9 hours in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should only claim those hours he/she spent in the educational activity.

Course Evaluation

It is NASPGN policy to conduct post activity evaluations. The results of these evaluations are used to plan future CME activities and are shared with the faculty. Please complete the evaluation and return it to the Registration Desk. Evaluations must be completed in order to receive your CME verification.

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Current ACCME guidelines state that participants in CME activities should be made aware of any affiliation or financial interest that may affect the speaker's presentation(s). Therefore, it is the policy of the North American Society for Pediatric Gastroenterology and Nutrition (NASPGN) to insure balance, independence, objectivity, and scientific rigor in all its educational programs. All faculty participating in any NASPGN sponsored program are expected to disclose to the program audience any real or apparent conflict(s) of interest that may have a direct bearing on the subject matter of the continuing education program. This pertains to relationships with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of the presentation topic. The intent of this policy is not to prevent a speaker with a potential conflict of interest from making a presentation. It is merely intended that any potential conflict should be identified openly so that the listener may form his own judgment about the presentation with the full disclosure of the facts. It remains for the audience to determine whether the speaker's outside interests may reflect a possible bias in either the exposition or the conclusions presented.

Scheduled speakers for NASPGN Postgraduate Course have provided the following information, which is accurate at the time of printing.

Faculty Member	**Relationship identified with**
William F. Balistreri	No Relationship to Disclose
Bruce R. Bacon	No Relationship to Disclose
Linda Book	No Relationship to Disclose
Jane Brock	No Relationship to Disclose
Mei-Hwei Chang	No Relationship to Disclose
Carlo DiLorenzo	No Relationship to Disclose
Peter Durie	Research Grants: Organon Canada, Digestive Care Inc. and Solvay Pharma
Edward J. Hoffenberg	No Relationship to Disclose
Maureen M. Jonas	No Relationship to Disclose
Lynn B. Jorde	No Relationship to Disclose
Lee Kaplan	No Relationship to Disclose
Deirdre Kelly	No Relationship to Disclose
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Piero Rinaldo	No Relationship to Disclose
Joel Rosh	No Relationship to Disclose
Scott H. Sicherer	No Relationship to Disclose
Richard Schreiber	No Relationship to Disclose
Ernest Seidman	Consultant & Member of Speaker's Bureau – Prometheus Labs
Doris B. Strader	No Relationship to Disclose

Signed Disclosure(s) are on file at the NASPGN Offices.

Notes

Basic principles of screening

Jane Brock, MD, MSPH

LEARNING OBJECTIVES

At the conclusion of this session, participants will be able to:

1. Decide when a screening test is applicable;
2. Evaluate test properties: sensitivity, specificity, ROC curves;
3. Interpret screening test results for an individual patient: predictive value;
4. Interpret results of a test in populations with different incidences of disease.

Basic Principles of Screening

Jane Brock, MD, MSPH

1. Screening: What is it?

Screening is the PRESUMPTIVE identification of UNRECOGNIZED disease by using a TEST which is RAPIDLY applicable to sort out WELL persons who PROBABLY DO have the disease from those who DO NOT have the disease.

2. Screening: When should we use it? (Table 1)

There is a significant ethical difference between everyday medical practice and screening. Initiating a screening procedure should follow conclusive evidence that screening can alter the natural history of disease in a significant proportion of those screened.

Answer the following questions:

2.1. If the condition is found, how effective is the ensuing treatment in terms of:

- Efficacy
- Patient compliance
- Lead time (time between detection of asymptomatic disease by the screening test and actual diagnosis by appearance of symptoms): if lead time is short, there may not be an advantage in screening
- Practical issues: are there enough resources, locations, materials, providers, etc. to respond to patients with positive tests

2.2. How great is the burden of suffering caused by the condition in terms of:

- mortality
- disability
- discomfort
- financial costs

2.3. How good is the screening procedure in terms of:

- sensitivity
- specificity
- simplicity
- cost
- safety
- acceptability by patients

Health care providers are more trained to consider the consequences of false-negative tests (the disease treatment will be delayed and poor outcomes are more likely). However, the consequences of false positives can be equally devastating. Patients can pay high emotional and financial costs, and may gain a 'label' which can be difficult to remove even after subsequent testing shows they do not in fact have the disease. False positives also pose a major burden to the health care system in that a large group of people needs to be brought back for further testing, when only a few of them may turn out to have the disease.

3. Screening: What is a normal (as opposed to abnormal) test?

3.1. The Normal Bell-Gaussian Curve (Table 2)

3.2. Using Cutoffs

A GOLD STANDARD is absolutely needed here. It must be something tangible and widely accepted to define absence or presence of disease. Then, the screening test can be compared to the gold standard. The more definitive the gold standard is the better your comparison will be. (Table 3)

4. Evaluating the test

Now that you have defined the "normal" test (= rule out disease), how do you evaluate the test? The test needs to be able to change the pre-test probability of having the disease. The PRE-TEST PROBABILITY of having the disease corresponds to the prevalence of the disease among the tested population; in other words, it is the probability that an undiagnosed patient has the disease when he/she comes to your office, before any test is done. (Table 4)

4.1. SENSITIVITY: The ability to correctly identify those WITH the disease or A/(A+C)

4.2. SPECIFICITY: The ability to correctly identify those WITHOUT the disease or D/(B+D)

4.3. ROC curves (Receiver-operating-characteristics curves)

Not only is this is a way to evaluate the test but also when a test can have several possible cutoffs, this is a way to determine the best cutoff. To construct a ROC curve, for each possible cutoff of the test, the values of sensitivity and the values of (1-specificity) are plotted as shown below. Using the example of CPb in lead poisoning (data are artificial), see Table 5 for a

ROC curve.

ROC curves are used to evaluate tests. The larger the area under the curve, the better the test. This is quite often used to compare tests by comparing the areas under their ROC curves.

In addition, ROC curves are used to determine the best cutoff when such a threshold is desired. Providers and patients are often more comfortable thinking about and discussing test results described as categorical variables (so do I have the disease or not?). It is important to recognize, however, that test results are almost always continuous variables which have been dichotomized for ease of use. What a positive result really represents is an increased probability (chance) of having the disease. The best cutoff corresponds to the point closest to the left upper corner of the curve. This is the point farthest away from the worst possible results represented by the diagonal line. As you can see, the point in the left upper corner has the best combination of sensitivity and specificity.

5. Predictive Value: How does the test result apply to my patient?

5.3.1. PREDICTIVE VALUE OF A POSITIVE TEST: The proportion of true positives among all positive tests or A/(A + B)

5.3.2. PREDICTIVE VALUE OF A NEGATIVE TEST: The proportion of true negatives among all negative tests or D/(C + D)

Often the purpose of testing is to increase the estimate of probability high enough to begin treating the patient, or decrease the estimate low enough to consider the disease ruled out. The predictive value of any test is directly influenced by the prevalence of the condition being tested for in the population being tested.

Pre-test probability – An estimate of the probability of disease based on information available prior to testing. In the absence of any specific information about the patient, the pre-test probability equals the prevalence/incidence of disease in the population.

Post-test probability – A revised estimate of the probability of disease after the test results are known.

Example: Screening all children in a community for lead poisoning by using samples obtained by fingerstick (CPb). The prevalence of lead poisoning in a given moderately exposed community is 5%; thus, pre-test probability is 5%.

Note that sensitivity of test (A/(A+C)=30/50) is =60% and specificity (B/(B+D)=864/950) is =91% (Table 5)

Let's now change the pre-test probability by looking at a population referred to the leading specialist in the nation on lead poisoning, where the incidence of the disease is much higher. In other words, this phenomenon occurs when a test is evaluated in the general population (where the incidence is low) and then in population referred to a specialist because of risk factors of some sort (where the incidence is much higher). (Table 7)

Note that sensitivity (540/900) is still 60% and specificity is still 91%. Sensitivity and specificity are test properties and are not affected by the disease incidence. Predictive values, however, are related to the incidence of the disease.

6. Likelihood ratio – With what degree of certainty do I believe this test? (Tables 6 and 7)

In contrast to sensitivity and specificity, interval likelihood ratios capture the magnitude of abnormality of test results.

Likelihood Ratio of a Positive Test: True Positive Rate/ False Positive Rate or A/(A+C) DIVIDED BY B/(B+D)

A person with a positive test is "x" times more likely to have the disease than NOT to have the disease.

Likelihood Ratio of a Negative Test: False Negative Rate/ True Negative Rate or

A person with a negative test is "x" times as likely to have the disease as NOT to have the disease.

7. Screening: parallel vs. serial testing (Table 8)

8. The risk of a false positive or "life is tough"

Relation between number of tests ordered and % of Normal people with at least one abnormal test result.

Table 9

No. of tests	People with at least one abnormal test
1	5%
5	23%
20	64%
100	99.4%

9. Outcome evaluation: three types of bias plague screening evaluations

9.1. Lead time bias:

Screening may decrease lead time (time between detection of asymptomatic disease through screening and time of diagnosis due to symptoms) without altering time to outcome, either because the treatment for the disease is not effective/non-existent or the screening test has poor sensitivity.

Individual not screened:

————————————symptoms———outcome

Individual screened:

—test—symptoms————————outcome

9.2. Length bias or increase in pre-clinical stage:

Patients who are screened and test positive become more aware of potential symptoms.

Individuals with disease screened:

test +——symptoms————————outcome

test - ————————symptoms————outcome

9.3. Self-selection bias:

Screened patients are different from non-screened patients. This happens quite commonly in studies done in referral centers.

10. Screening: a last word about it

" ...physicians must be content to end not in certainties, but rather in statistical probabilities. The modern physician thus has a right to feel certain, within statistical constraints, but never cocksure. Absolute certainty remains for some theologians …and like-minded physicians."

— David Spodick, MD

References

1. Sacket D L, Haynes RB, Tugwell P. Clinical Epidemiology, 2nd. Ed. Little, Brown and Company, Boston, 1991.
2. Fletcher RH, Fletcher SW, Wagner EH. Clinical Epidemiology: the Essentials, 3rd ed. Williams & Wilkins, Baltimore, 1988.
3. Gordis, L. Epidemiology 1st ed. W.B. Saunders Co., Philadelphia, 1996.
4. Gehlbach SH. Interpreting the medical literature. 3rd ed. McGraw-Hill, Inc. NY, 1993.
5. Barratt A, Irwig L, Guyatt GH, et al. The Users' Guides to the Medical Literature: XVII. How to use Guidelines and Recommendations About Screening. JAMA 1999; 281:2029-2035.

Table 1

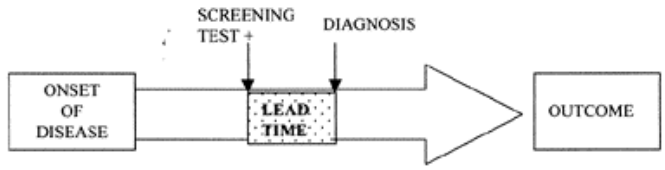

Table 2

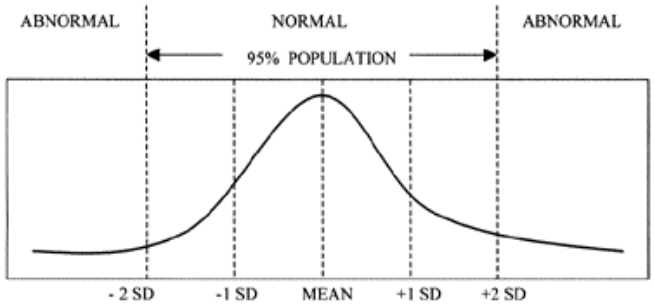

Table 3

Example: Use of Capillary Tube Blood Test (CPb) in Diagnosis of Lead Poisoning

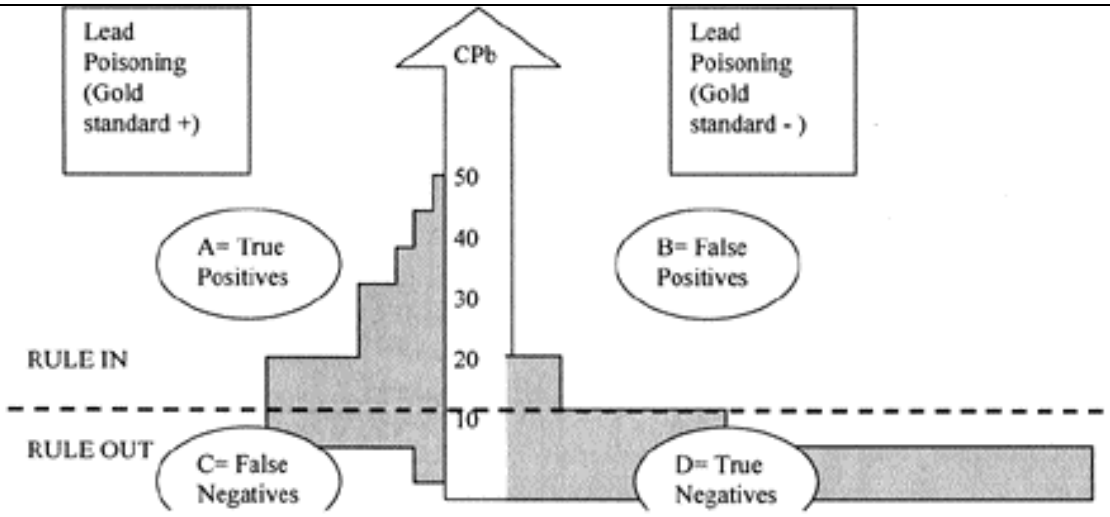

Table 4

		Lead Poisoning	
		Present (Gold Standard +)	Absent (Gold Standard -)
TEST CPb Result	Positive (>=10 ug/dl)	A = True positives	B = False Positives
	Negative (<10 ug/dl)	C = False negatives	D = True Negatives

Table 5

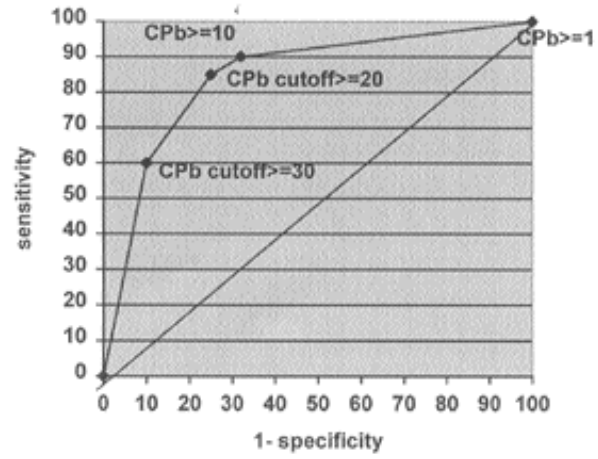

Table 6

		Lead Poisoning		
		Present (Gold Standard +)	Absent (Gold Standard -)	
TEST CPb	Positive	A = 30	B = 86	116
	Negative	C = 20	D = 864	884
		50	950	1000

Pretest Probability (or incidence of disease)	POSTTEST PROBABILITY (or predictive value of test)	CHANGE FROM PRETEST PROBABILITY
1%	Positive test=30/116=26%	+21%
	Negative test=100%- 864/884=2%	- 3%

Table 7

		Lead Poisoning		
		Present (Gold Standard +)	Absent (Gold Standard -)	
TEST CPb	Positive	A = 540	B = 9	549
	Negative	C = 360	D = 91	451
		900	100	1000

Pretest Probability (or incidence of disease)	POSTTEST PROBABILITY (or predictive value of test)	CHANGE FROM PRETEST PROBABILITY
90%	Positive test=540/590=98%	+8%
	Negative test=100%- 91/451=80%	- 10%

Table 8

	Parallel tests	Serial tests
Indications	rapid assessment (referral center)	-time is not an issue -expensive/ risky test
Sensitivity Predictive Value of Negative Test	↑	↓
Specificity Predictive Value of Negative Test	↓	↑

Screening for celiac disease

Edward J. Hoffenberg, MD

LEARNING OBJECTIVES:

At the conclusion of this session, participants will:

1. Know the conditions associated with celiac disease, and the incidence in the population;

2. Understand the potential benefits and limitations of screening for celiac disease;

3. Know what tissue transglutaminase antibody testing is about.

Screening for Celiac Disease

Edward J. Hoffenberg, MD

Screening tests for celiac disease

Non-serologic testing

Non-serologic tests that may identify individuals at risk for CD include genotyping tests and tests for mucosal integrity. Genotyping using cord blood has been a strategy we have used in stratifying the risk for developing celiac disease in newborns. Subgroups have been followed prospectively for the first several years of life. Newborns who express DQ2 or DQ8 represent about 30% of the population in Denver, but > 90% of the celiac disease population. DQ2 testing is available commercially, but is not specific. Other tests include D-xylose, breath hydrogen, and lactulose/mannitol tests. These tests are neither sensitive nor specific.

Serologic testing

A variety of serologic tests are available. As shown in Table 1, the sensitivity and specificity are variable. This is in part due to the background prevalence of CD in various populations studied, poorly standardized test techniques, and the subjectivity inherent in interpreting immunofluorescent assays. In addition, most serologic tests for CD are IgA tests, and IgA deficiency is more common in CD. One strategy has been to begin with an IgA test (endomysial, or tTG assays) and IgG gliadin assay. Those with only a positive IgG assay should have total IgA measured. Small bowel biopsy is performed on those with any positive IgA antibody and those who are IgA deficient with IgG anti-gliadin antibodies. Alternatively, any symptomatic IgA deficient individual should undergo biopsy.

Table 1

Serologic Tests for Celiac Disease (Adapted from [14])

	Sensitivity	Specificity	Comments	Cost Range Denver US$
Gliadin				
IgA	31-100	85-100	ELISA	45-85
IgG	46-100	67-100	ELISA	45-84
Reticulin	29-100	95-100	IFA	46
Endomysium	57-100	95-100	IFA	65-125
Transglutaminase (little data in young children)	?-100	?-100	ELISA, RA	95-130

Transglutaminase

The newest test for celiac disease is the IgA tissue transglutaminase (tTG) antibody assay. Transglutaminase is found in the extracellular matrix in the jejunum as well as other sites, including skin, liver, and red cells. The transglutaminase family of enzymes catalyze protein cross-linking in a calcium-dependent reaction forming gamma-glutamyl-lysine bonds. Dieterich et al identified tTG as the target recognized by anti-endomysial antibodies.[1] Subsequently, assays using guinea pig transglutaminase, and more recently human transglutaminase, have been developed, as EIA or radioassays. The EIA is widely commercially available. A number of reports consistently show that the tTG and endomysial assays provide similar results.

Advantages of the tTG assay include its: 1) reproducibility; 2) technical simplicity; and 3) lack of dependence on monkey esophagus or other tissue. The limitations of this assay are: 1) inaccuracy in IgA deficiency; 2) limited data in very young children; and 3) limited data in monitoring the GFD.

Two new papers show that tTG acts on A-gliadin to create an immunodominant epitope that elicits a strong immune response.[2,3] The peptide is from position 60-70: PFPQPQ(® E) LPYPQ, the bold Q is de-amidated to glutamate, represented by E. tTG catalyzes the de-amidation of the glutamine residue to glutamate at position 65 of A-gliadin. It is theorized that this moiety then binds to DQ2, induces a conformational change and an increased affinity for the DQ2 receptor. The underlined sequence represents the smallest sequence common to both papers. If a specific peptide sequence does induce the immune response in most CD patients, then this provides a focus for strategies to induce tolerance and to genetically alter wheat.

In summary, the tTG assay is a useful test for screening individuals with CD, and might replace some of the older assays. Why these antibodies develop in CD is unclear. Perhaps these antibodies play a role in the pathogenesis of CD.

Indications for using celiac disease screening tests

Clinical signs or symptoms

Celiac disease (CD) is a genetic and immunologic disorder characterized by intestinal injury upon ingestion of wheat proteins and related cereals. Based on seropositivity rates of approximately 1 in 250 in the United States[4] and as high as 1 in 100 in Europe,[5] CD is both common and under diagnosed. Signs and symptoms suggestive of CD are multiple, diverse, and non-specific (Table 2). Therefore, the clinician needs to keep CD in the differential diagnosis of a myriad of presentations. Hill et al found that 2-3% of children seen in an outpatient gastroenterology clinic setting for a variety of complaints had CD.[6] However, few would agree that screening every child with abdominal pain for CD is a cost-effective strategy. Hence, the challenge is to select appropriate patients for CD screening. Unfortunately, clear guidelines do not exist, and the perfect screening test remains to be developed.

For every known CD patient there are an estimated 5-7 undiagnosed and presumably “asymptomatic” individuals with CD.[7] These “asymptomatic” individuals may be labeled as having

Table 2

Selected Presentations of Celiac Disease (Adapted from [14])

	Traditional	Non-traditional or emerging
Common		
	Chronic diarrhea	No symptoms
	Failure to thrive	Pruritus/dermatitis herpetiformis
	Irritability	Neurologic epilepsy, cerebral calcification[15]
	Abdominal distention, bloating	ataxia[16]
	Weight loss (older child, adult)	dementia, seizures
	Weakness (older child, adult)	Dental enamel defects[17]
		Osteoporosis/osteopenia[18]
		Arthritis
Less Common		
	Constipation	
	Vomiting alone	
	Dyspepsia, indigestion (older child, adult)	
	Recurrent abdominal pain (older child, adult)	
	Pubertal delay (older child, adult)	
	Short stature/growth delay (older child, adult)	
	Iron deficiency, folate, calcium, or vitamin K deficiency	

"silent" (no symptoms), "subclinical" (mild, not previously considered by patient as significant) or "latent" (CD that manifests with gluten challenge or with time). Most feel healthy and do not seek medical attention. Like "classic" or symptomatic patients, those with the "asymptomatic" form of CD generally have circulating CD-associated antibodies, and characteristic intestinal villus atrophy with increased numbers of inter-epithelial lymphocytes.

The term "latent" CD has been applied to those who have positive antibody tests, but small bowel mucosa that is normal or shows only increased numbers of inter-epithelial lymphocytes. Small bowel injury and or symptoms develop with time or with gluten challenge.

To make the initial diagnosis of CD, the experts I polled still use the revised ESPGHAN criteria, requiring villus atrophy while on a gluten-containing diet.[8] The presence of CD-associated antibodies is not considered sufficient for the diagnosis of CD.

Genetic risk for celiac disease (associated diseases, family history)

CD occurs more frequently in certain groups, as shown in Table 3. The closest relationship is with dermatitis herpetiformis, which may be considered an extra-intestinal manifestation of celiac disease. Other autoimmune disorders associated with CD include type I diabetes, Sjogren's syndrome, and IgA deficiency. Trisomy 21 has been associated with celiac disease, although the mechanism is unclear.

Management of known CD patients

Many clinicians use follow-up measurement of CD-associated antibodies in known patients following a GFD. The main reasons are: 1) to support the diagnosis of CD by demonstrating decrease in titer with gluten elimination, and rise in titer with gluten challenge; and 2) to assess compliance with GFD (detect unsuspected or suspected gluten ingestion).

Table 3

Groups at Risk for Celiac Disease Include:

Risk Factor	Frequency of CD
Dermatitis Herpetiformis	+/- 100%
Type 1 diabetes	5-10% [19]
IgA deficiency	7%[20]
Down Syndrome	7% to 19%[21, 22]
Sjogren	3-15%[23]
First degree relative with CD	4 to 11%[24]
Second degree relative	5% (Berti, et al)
Juvenile chronic arthritis	2.5%[25]
Autoimmune thyroid disease	8% (2000, WCG abstract)
GI clinic and risk or symptoms	2-3%[6]

New data in newly diagnosed pediatric CD suggest that high tTG titers correlate with more severe mucosal injury.[9] On the other hand, there are data showing that disappearance of endomysial antibodies does not correlate with mucosal healing.[10] There are no published data yet on the use of tTG titers for monitoring compliance with the GFD.

Population studies

Population screening for CD is attractive for many reasons. CD is common; gluten is universal in the Western diet; tTG antibody screening can be performed on large numbers of samples; identifying CD before onset of symptoms might reduce health care costs; at-risk groups are defined, an effective treatment is widely available; and GFD has been shown to decrease the long-term risk of CD-associated malignancy.[11]

However limitations to mass screening also merit consideration. One issue is the potentially serious emotional and social implications of identifying an asymptomatic child as having a chronic disease requiring lifelong treatment. There is the potential for difficulty in obtaining health and life insurance and for other forms of discrimination.

Another concern is that many aspects of the natural history of celiac disease are unknown. For example, we do not understand the natural history of "asymptomatic" CD, specifically the long term risks of delay in diagnosis and treatment. Preliminary evidence implies that longer exposure to gluten is associated with a greater risk for other autoimmune diseases, such as thyroiditis and alopecia.[12] Although it seems reasonable to assume that "asymptomatic" CD and "symptomatic" CD confer a similar risk for malignancy, and that a GFD provides similar benefit, there are limited data to support these assumptions.

Another concern is our poor understanding of the pathogenesis of CD. Why does autoimmunity develop in a few, but tolerance in most, at-risk individuals? What influences the timing of onset of disease (infancy, childhood, adulthood) and the severity of disease (asymptomatic vs symptomatic)? Why do tTG antibodies develop? What is the mechanism of small bowel injury? These issues are important in planning screening strategies. Most

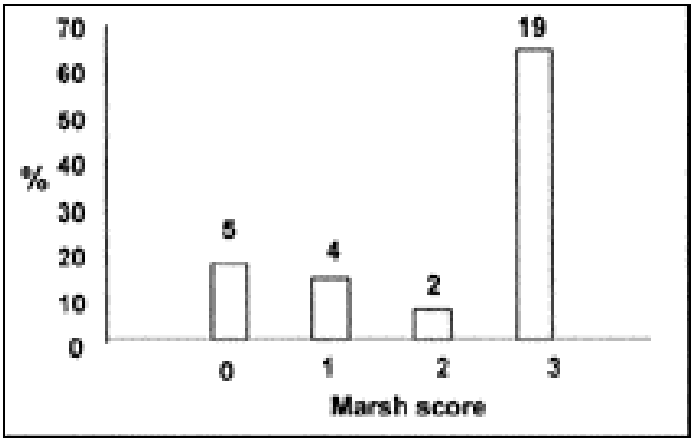

Figure 1
Distribution of small bowel biopsy histology score using system of Marsh in 30 children screened because of a genetic risk for celiac disease.[9] Marsh score of 0 indicates no evidence of celiac disease, 1 indicates increased intra-epithelial lymphocytes, 2 indicates enlarged crypts, 3 indicates subtotal or total villus atrophy.

reports of population screening show that virtually all seropositive individuals (usually adolescents and adults) undergoing biopsy do show mucosal injury consistent with CD. This has led some to suggest that a positive antibody test (endomysial or tTG) might be sufficient for the diagnosis of CD, and that small bowel biopsy might not be necessary. In contrast, our data show that about 20% of seropositive young children screened solely because of a genetic risk of CD currently show NO biopsy evidence of CD (Figure 1).[9] Whether these children go on to develop CD is unknown. An alternative explanation might be that these patients develop a transient seropositive stage on the way to developing tolerance. This would profoundly alter the interpretation of a single positive test for CD.

Finally, strategies that involve screening children must acknowledge that most adolescents with CD do not comply with a strict gluten-free diet,[1 3] mitigating potential benefits of screening. Indeed, any "asymptomatic" CD patient is more likely to be non-compliant with the GFD than a symptomatic patient is.

My personal conclusion is that the technology for population screening is available, but that it should remain a research tool until there is a more clear understanding of the natural history and pathogenesis of "asymptomatic" CD, a careful analysis of the implications (medical, psycho-social, cost-benefit) of screening, and better treatment options become available (easier GFD, ability to induce tolerance, etc.).

Take home messages

1. Remarkable advances in the ability to screen for CD are leading to new knowledge of the pathogenesis of CD.

2. Major advances include

   a. identification of tTG as a major antigen in CD that is useful as a screening tool.

   b. focus on how tTG relates to autoimmunity and the pathogenesis of CD.

   c. recognition of the high incidence of "asymptomatic" celiac disease in Europe and the Americas.

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Notes

Screening for inflammatory bowel disease

Ernest Seidman, MD, FRCP

LEARNING OBJECTIVES

The overall objectives of this presentation are to review the indications, clinical accuracy and limitations of non-invasive screening tests for IBD in the pediatric age group. The participant will be able to:

1. Understand the basis and technological aspects of pANCA and ASCA serological assays;

2. Appreciate the clinical utility of these tests in terms of screening for IBD, as well as in distinguishing between types of IBD;

3. Become familiar with the role of other non-invasive tests to screen for IBD as well as determine disease activity, such as fecal markers and color Doppler ultrasound.

Screening for Inflammatory Bowel Disease

Ernest G. Seidman, MD

Summary

The diagnosis of IBD is generally based on standard clinical, endoscopic, histologic and radiographic criteria. In the pediatric age group, there is often considerable delay in diagnosis, particularly for Crohn's disease. There is thus considerable interest in finding an accurate diagnostic marker that would enable clinicians to diagnose or to rule out IBD with certainty, in a noninvasive manner. The challenge is to find a marker or combination of markers that possess the specificity and sensitivity criteria necessary to accurately screen for IBD. In addition to discriminating IBD from non-IBD, another diagnostic challenge facing clinicians is to accurately differentiate Crohn's disease (CD) from ulcerative colitis (UC) when the disease is restricted to the large bowel. This presentation summarizes recent advances using non-invasive diagnostic markers for screening for IBD, as well as for differentiating between types of IBD.

Screening for IBD

Serological assays

Recent studies have attempted to validate the clinical utility of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and anti-saccharomyces cerevisiae antibodies (ASCA) in IBD. pANCA is an autoantibody against a specific neutrophil antigen that is quite specific for UC, whereas ASCA is highly specific for CD, making these antibodies ideal candidate markers for the differentiation of UC from CD. In the Ste Justine Hospital study,[1] IgA or IgG ASCA was present in 55% of CD patients, yet detectable in < 5% of UC and non-IBD patients (Table 1). When ASCA IgA and IgG antibodies were both positive, the assays were 100% predictive of CD. This data suggests that ASCA is highly specific and predictive for CD. Although present in 57% of UC patients, pANCA was also detected in 13% of CD patients. Interestingly, the majority of these CD patients had a "UC-like" presentation. These findings suggest that pANCA may represent a particular IBD phenotype, perhaps an overlapping CD/UC subtype. We also examined whether these markers are helpful in cases of indeterminate colitis where the differentiation between CD and UC is particularly important when making decisions about surgical management. The diagnostic accuracy of these assays was thus evaluated among those patients with disease limited to the colon. ASCA IgA and IgG were 100% specific for CD and pANCA was 97% specific for UC, suggesting that the presence of these markers in patients with indeterminate colitis assists clinicians in discriminating between CD and UC with greater certainty. No significant correlation was observed between the presence of ASCA or pANCA and disease location, duration, activity, complications and therapeutics. Unlike the pANCA titers that remained elevated in patients with UC after colectomy, ASCA did not persist in a small group of CD patients after intestinal resection.

In another pediatric study (2), the highest sensitivity (71%) and specificity (84%) for IBD was obtained when the ANCA and ASCA assays were combined, rather than evaluated independently. To differentiate CD from UC, the more disease specific pANCA assay was employed. The specificity of pANCA for UC was only 65%, significantly lower than previous reports (Table 1). Although ANCA was present in some CD patients, titer levels > 100 EU/ml were specific for UC. This suggests that quantitative analysis of these assays may be useful for differentiating CD from UC. The authors concluded that the combination of ASCA and ANCA testing identified approximately 2/3 of their IBD patients. The presence of these markers among unaffected family members and their moderate sensitivity and specificity was felt to limit the utility of these antibodies for at-risk population screening.[2] Data from a preliminary study aimed to test the accuracy of these assays in screening for IBD in pediatric cases with possible functional or irritable bowel disease (IBS) look very promising.[3]

Despite using different technology and adult patient groups, a European study[4] reported similar sensitivity and specificity data of the individual assays (Table 1). There was no significant correlation observed between the presence of ASCA or pANCA and disease activity, treatment, or previous surgery. However, the prevalence of ASCA was significantly higher in patients with small bowel involvement (with or without colonic disease) and in patients diagnosed with CD at a younger age. In contrast to previous reports, there were no clinical features particular to pANCA+ CD patients in this study. When compared to individual assay performances, the combination of ASCA and pANCA serology appeared to maximize the predictive value of these tests in differentiating CD and UC. Additionally, very few patients with non-IBD colitis were positive, suggesting that these markers are highly predictive of IBD when the differentiation of diarrheal illnesses remains uncertain.

In a Japanese study,[5] immunoflourescence rather than ELISA was used to detect the presence of pANCA. Less than 3% of the non-IBD controls had detectable pANCA (Table 1). However, among this adult IBD cohort, pANCA was more prevalent among CD than UC patients (72% vs. 64%). A cytoplasmic staining pattern was observed in up to 15% of IBD patients. The authors concluded that ANCA is a useful marker to distinguish IBD from healthy controls. However, in contrast to the previous reports, it did not help distinguish UC from CD. It remains difficult to compare studies that evaluate the accuracy of these assays, given differences in methodology, disease prevalence, patient selection and definitions of disease. Further

studies are needed to determine if genetic or epidemiologic differences influence the antigenic stimulation of pANCA.

Fecal markers

Fine et al.[6] attempted to determine the causes of chronic diarrhea using a rapid latex agglutination technique detecting lactoferrin, a protein component of neutrophil granules. None of the normals and 100% of the confirmed IBD controls had a positive lactoferrin test. Among the unknown cases of diarrhea 12 of 103 were diagnosed with IBD and 11 of these had a positive fecal lactoferrin test. The sensitivity and specificity of fecal lactoferrin in this small sample of IBD patients was 90% and 98%, respectively (Table 1).

Imaging methods

Hollerbach et al.[7] conducted a prospective study to determine the clinical value of abdominal ultrasound (US) in the diagnostic assessment of inflammatory disorders (appendicitis, diverticultis and IBD) and their respective complications. US findings were compared with the final radiologic, endoscopic or pathologic diagnosis. The sensitivity was 84% and 66% for CD and UC, respectively (Table 1) with the overall specificity for all diagnoses was 95%. Ileal and colonic lesions were preferentially detected by US. Although US was highly predictive of an inflammatory bowel disorder, it was not disease specific. Andreoli et al.[8] evaluated the accuracy of ultrasound compared to endoscopy in the diagnosis of postoperative recurrence of Crohn's disease. Forty-one patients with an ileocolonic anastomosis underwent both ultrasound (US) and endoscopy within 2 weeks. Approximately 50% of patients had symptoms suggestive of a postoperative recurrence at the time of these studies. US recurrence was defined by an ileal wall thickness > 5-mm. The sensitivity and specificity of US in this study was 81% and 86%, respectively (Table 1). Due to the high positive predictive value (96%) of recurrence based on US alone, the authors predicted that nearly 65% of colonoscopies could have been eliminated in their study population. The authors therefore concluded that US should be performed first on all patients with suspected post operative recurrence and endoscopy only in the case of a negative US. More recently, we found that quantification of bowel mucosal vessel density by color Doppler US was able to differentiate active CD from inactive CD and non-IBD patients.[9] A larger prospective studies is currently underway to determine the clinical utility of non-invasive Doppler US in the follow-up of CD after therapy, in order to determine its accuracy in predicting relapse.

Conclusion

The search for a non-invasive diagnostic marker in IBD is emerging as an important area of research among clinical investigators. Evaluating the cost-effectiveness of these various non-invasive markers will be the next challenge to be faced.

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Table 1

Test Characteristics of Various Diagnostic Markers in Inflammatory Bowel Disease*

Study	n	Diagnostic Marker	Test Population	Sensitivity (%)	Specificity (%)
Serological Markers					
Ruemmele et al.	209	ASCA	CD vs. UC & controls	55	95
		pANCA UC	UC vs. CD & controls	57	92
Hoffenberg et al.	119	ASCA	CD vs. UC	60	88
		pANCA UC	UC vs. CD	60	65
Quinton et al.	391	ASCA	CD vs. UC	61	88
		pANCA UC	UC vs. CD	65	85
Sugi et al.	169	pANCA	CD&UC vs. controls	67	97
Fecal Markers					
Fine et al.	154	Lactoferrin	CD&UC vs. controls	90	98
Radiological Markers					
Hollerbach et al.	227	Abdominal ultrasound	CD UC	84 66	not available
Andreoli et al.	51	Abdominal ulstrasound	CD vs. controls	81	86

*Adapted from: Dubinsky MC, Seidman EG. Diagnostic markers of inflammatory bowel disease. Current Opinion in Gastroenterology, 2000; (in press).

Screening for cystic fibrosis and hereditary pancreatitis

Peter R. Durie, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will gain an understanding of:

1. The clinical spectrum of cystic fibrosis and hereditary pancreatitis;
2. The molecular basis of cystic fibrosis and hereditary pancreatitis;
3. Screening and diagnostic methods for cystic fibrosis and hereditary pancreatitis.

Screening for Cystic Fibrosis and Hereditary Pancreatitis

Peter R. Durie, MD, FRCPC

Introduction

Diseases of the exocrine pancreas are one of the most difficult diseases to investigate and treat. The inaccessible location of the gland, its propensity to become destroyed and the lack of specific and sensitive functional tests contribute greatly to this challenging problem. Recent advances in molecular genetics and the opportunity for direct genetic testing provide an opportunity to establish a diagnosis in affected patients and family members. Nevertheless, the advent of genetic testing creates a greater level of complexity for the clinician and may result in more uncertainty for the individual patient. Before patients are offered molecular testing, the risks and benefits must be carefully assessed. Without proper safeguards and appropriate counselling, genetic information can have harmful consequences to the patient, which may include psychologic damage and negative effects on employability and health and life insurance.

Cystic fibrosis

Many of the manifestations of CF are due to an inability of secretory lumina to hydrate and solubilize macromolecules. Mucus secretions in the bronchi and the intestines are viscid and inspissated. Certain organs such as the pancreas and the vas deferens are the most vulnerable to flow related problems because of the tortuosity and the length of their ducts and the high macromolecule concentration of their secretions. Fluid secretion in most epithelia are dependent upon the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cAMP responsive chloride channel. Thus, deficient fluid secretion due to loss of CFTR, or its dysfunction, may induce organ damage as a result of gelled or precipitated macromolecules causing ductal or glandular plugging. Absence of a secretory protein or other macromolecule within glands (eg. the sweat duct), probably protects it from pathologic damage.

The cDNA product of the CF gene was cloned and sequenced in 1989. The primary amino acid sequence of the gene product suggested a similarity to other membrane transporters, due to the presence of two membrane spanning domains, separated by two nucleotide binding domains and a unique intervening regulatory domain (R-domain). The most common CFTR gene mutation causes a deletion of a single phenylalanine molecule at position 508 of the 1480-amino acid CFTR protein. This gene mutation (DF508) occurs in 69% of CF chromosomes worldwide. More than 900 additional CFTR gene mutations have since been described, but most of them are infrequent or rare.

The age at which symptoms first occur, the degree of involvement and the severity of disease in affected organs, vary considerably from patient to patient. Patterns of clinical manifestations are almost certainly influenced by multiple factors including genetic, environmental and varying treatment strategies. It has been possible to analyze patient's genotypes and their corresponding phenotypes. Perhaps the most striking observation is the direct influence of genetic factors on the degree of pancreatic disease. Cross-sectional analysis of large CF populations reveal that 85-90% of patients have pancreatic insufficiency (PI) and require enzyme replacement therapy with meals. The remainder, who have pancreatic sufficiency (PS) do not need pancreatic enzyme supplements. The latter term is an operational one reflecting the ability of patients to digest food normally without enzyme supplements. All patients with the PS phenotype have abnormal pancreatic fluid and electrolyte secretion, and a large percentage have deficient enzyme secretion. As a group, CF patients with PS are diagnosed at a later age and experience milder symptoms, particularly with respect to lung disease. Most PS patients have abnormal sweat chloride concentrations but the concentrations are lower than those with PI.

Severe pancreatic disease is strongly associated with the presence of DF508; 99% of those homozygous for DF508 have PI. We have screened a large patient cohort for additional CFTR gene mutations. Almost all genotypes are associated only with PI or only with PS but not with both. On this basis, mutations have been classified as severe or mild with respect to pancreatic function status (Table). A severe mutation combined with any other severe mutation is associated with PI. In

Table

Classification of CF Gene Mutations as "Severe" or "Mild" with Respect to Pancreatic Function

Type of Mutation	Severe (location)	Mild (location)
Missense (point mutation)	1148T (exon 4) G480C (exon 9) V520F (exon 10) G551D (exon 11) R560T (exon 11) N1303K (exon 21)	R117H (exon 4) R334W (exon 7) R347P (exon 7) A455E (exon 9) P574H (exon 12)
Single amino acid deletion	DF508 (exon 10) DI507 (exon 10)	
Stop codon (nonsense)	Q493X (exon 10) G542X (exon 11) R553X (exon 11) W1282X (exon 20	
Splice junction	621+1G®T (intron 4) 1717-1G®T (intron 10)	
Frameshift	556delA (exon 4) 3659delC (exon 19)	

contrast, DF508 (or any other severe mutation) when combined with the mild mutation (or two mild mutations) is invariably associated with PS. Thus, mild alleles have a dominant effect over a severe allele.

Correlations between mutations and other phenotypic manifestations are less clear-cut. Meconium ileus appears to be strongly associated with severe PI mutations but with variable expression. It has been shown that modifier genes unrelated to the CFTR gene are responsible for the phenotypic expression of meconium ileus. CF-associated liver disease is more likely to be present in patients with severe PI mutations. The severity of lung disease and its rate of progression are less easily defined by genotype. For example, patients of the same age and sex who carry DF508 on both alleles can have widely variable lung function from normal to severely impaired. The tremendous variability in severity of lung disease in both patient groups suggest that other factors including environmental, infectious and possibly other genetic modifiers unrelated to the CFTR gene may influence the severity and progression of lung disease.

The ability to detect CFTR gene mutation has led to the recognition that the spectrum of the CF phenotype is much broader than previously believed. Approximately 2% of patients present with an "atypical" phenotype which include individuals with chronic sinopulmonary disease, pancreatic sufficiency plus either a borderline or normal sweat chloride concentration. In addition, there are patients in whom a single clinical feature suggestive of CF disease is apparent. This includes patients with an abnormal sweat chloride measurement without clinical manifestations of CF disease, or those that have pancreatitis, liver disease, sinusitis or obstructive azoospermia (males only) as the sole manifestation of disease. Recent advances in the ability to detect a large number of mutations has led to extensive screening of patients with monosymptomatic diseases with similarities to CF. For example, it is now recognized that a large percentage of patients with obstructive azoospermia, idiopathic pancreatitis, and atypical sinopulmonary disease, have a higher frequency of CFTR gene mutations than the general population. These observations have created considerable debate about where to draw the line between a confirmed diagnosis of CF and conditions in which molecular defects in the CFTR gene are identified. Regardless of where the diagnostic line is drawn, the existence of atypical manifestations of disease greatly extends the continuum of the clinical spectrum associated with defects in the CFTR gene.

Criteria for the diagnosis of CF

On the basis of a recent consensus report, the diagnosis of CF is established by:

One or more characteristic features of CF, a history of CF in a sibling, or a positive newborn screening test result, ***plus*** laboratory evidence of a CFTR abnormality (elevated sweat chloride or identification of two mutations in the CFTR gene known to cause CF*), or demonstration of characteristic abnormalities of ion transport in the nasal epithelium.

Screening for cystic fibrosis

Prenatal

In utero diagnosis of CF, based upon detection of two CFTR gene mutations in the fetus is now being made with an appreciable frequency. This form of testing is usually carried out in a family that has had a previously affected child or because the detection of fetal echogenic bowel on routine ultrasonography. Prenatal testing requires prior knowledge of both parental CFTR gene mutations.

Newborn

A number of regional and national newborn screening programs have existed for up to two decades. Strategies for newborn screening vary somewhat from program to program, but rely upon identification of an elevated immunoreactive trypsinogen concentration above a predetermined level of discrimination. Usually a second sample is requested and in some centers a repeat trypsinogen determination is carried out in conjunction with mutation analysis for the most common CFTR gene mutations. Confirmation of the diagnosis by sweat testing is required.

There are several arguments both in favor and against newborn screening for CF. Proponents of screening argue that early diagnosis leads to avoidance of signs and symptoms of disease, particularly morbidity and mortality associated with malnutrition. Detractors of newborn screening argue that there are an unacceptably high percentage of false positive results which creates considerable anxiety in parents of newborn children. Secondly, since there is no specific therapy for CF, other than symptomatic care, there is no convincing evidence that long term prognosis and survival is altered by early diagnosis.

Symptomatic diagnosis

Recognition that CF can present with a much milder phenotype creates a diagnostic challenge for the clinician. A high index of suspicion must be maintained since symptoms can be subtle and can occur at any age including in adulthood.

Sweat testing

Sweat chloride determination remains the mainstay for establishing the diagnosis of CF. It should be performed in an accredited CF center by pilocarpine iontophoresis. Two positive sweat tests (>60mmol/L) confirm the diagnosis. If a borderline result (40-60 mmol/L) is obtained, further sweat testing is recommended. Some mutations, for example 3849+10 kb C®T frequently results in a normal or borderline sweat chloride determination. Furthermore, patients with the PS phenotype have lower sweat chloride concentrations than those with the PI phenotype. Occasionally, values are within the borderline range. A sweat chloride concentration greater than 160 mmol/L is a physiologic impossibility. Such values suggest a technical error (collection or analysis of sweat) or a factitious result.

Genotype analysis

The presence of two mutations known to cause CF, predicts

* Only a limited number of the more than 900 known CFTR gene mutations are proven disease causing mutations.

with a high degree of certainty, that an individual has CF. Unfortunately, only a limited number of the known mutations (>900) are designated as CF disease-causing mutations. To be so designated, they must fulfill at least one of the following criteria:.

Cause a change in the amino acid sequence of CFTR that severely affects synthesis and/or function

Introduces a premature termination signal

Alters the nucleotides of the intron splice sites

Causes a novel amino acid sequence that does not occur in normal CFTR genes and has been identified in at least 100 carriers of CF patients.

At present, commercial laboratories only test for a limited number (30-75) of known CFTR gene mutations. With further advances in molecular technology, it is anticipated that a much larger panel of mutant alleles will be identified. Even when complete mutation analysis is performed in patients with a known diagnosis of CF, mutations remain unidentified in up to 10% of genes.

In conclusion, confirmation of the diagnosis of CF on the basis of mutation analysis is highly specific but not very sensitive. Some CF mutations occur with increased frequency, or even uniquely, in specific populations such as Ashkenazi Jews, African Americans and patients emanating from Northeastern Europe. Thus, mutation panels can be customized to the patient populations' ethnic background.

Nasal potential difference measurements

Airway epithelial surfaces, including the nasal epithelium, regulate the composition of fluids that bathe airway surfaces. This is achieved primarily by transport of sodium and chloride. Active transport of ions generate a potential difference (PD) across the epithelial surface which can be measured in vivo. Patients with CF have a different pattern of nasal PD than epithelia from unaffected patients. Three features of this test distinguish CF from normal individuals:

Raised basal PD which reflects enhanced sodium transport

Greater inhibition (%) of PD after superfusion of the epithelium with the sodium channel inhibitor amiloride.

Little or no change in PD following superfusion of the epithelial surface with a chloride free solution in conjunction with isoproterenol (cAMP agonist of CFTR).

Nasal PD measurements may assist in the diagnosis or exclusion of cases with CF, but certain considerations and precautions should be understood. The equipment must be safe and rigorously validated. The presence of nasal polyps or inflamed mucosa alters bioelectric properties and may yield false negative results. Performance and interpretation of PD measurements require a clear understanding of ion transport characteristics. The test is extremely difficult to perform and should only be accepted as a clinical diagnostic tool when sufficient numbers of studies have been performed (patients with CF, normal subjects and disease controls) and suitable reference values have been defined.

Ancillary tests of the CF phenotype

Several ancillary test of potential diagnostic should be considered.

Respiratory tract

Characterization of respiratory microbial flora can be helpful for diagnostic purposes due to the fact the CF respiratory tract has a predilection to the chronic colonization by Pseudomonas auruginosa, Staphylococcus aureus and Burkholderia cepacia.

Imaging studies (plain film and CT) of the lungs and paranasal sinuses may also be helpful in the diagnosis.

Exocrine pancreas

A number of direct and indirect tests (including blood test) are available to evaluate exocrine pancreatic function. Among the indirect tests, fecal fat analysis, with a timed pooled stool collection (minimum of 72 hours) is the most widely used and is probably the most informative. However, this test is of no value in assessing patients with PS. Direct tests, which are highly specific and capable of evaluating the entire range of pancreatic function are of great value for identifying aspects of pancreatic fluid and ion secretion in patients with a questionable diagnosis of CF. These tests, which involve naso-duodenal intubation and quantitative collection of stimulated secretion from the exocrine pancreas, require special skills to perform and interpret. Their invasive nature preclude their use for routine clinical purposes.

Adolescents and adults with idiopathic acute, recurrent or chronic pancreatitis carry a higher frequency of CFTR gene mutations than the general population. A subset of these patients fulfill the current diagnostic criteria for cystic fibrosis. Thus, full evaluation of patients with this presentation may be indicated. Some of these patients have no CFTR mutations. Others will carry one CFTR gene mutation or two mutations that are not proven to cause CF disease. Thus, in the majority of these patients the diagnosis of CF will not be confirmed by mutation analysis alone.

Urogenital evaluation

In post-pubertal males, infertility due to obstructive azoospermia is a highly consistent feature of the CF phenotype. This finding is present in approximately 98% of males with a confirmed diagnosis of CF and occurs as a result of absent or rudimentary vas deferens. Thus, careful evaluation of the genital tract in males in whom the diagnosis of CF is uncertain (palpation, semen analysis and transrectal ultrasound) may provide helpful diagnostic clues.

As well, individuals first seen in Andrology clinics because of infertility due to obstructive azoospermia may carry one or two CFTR gene mutations and have normal, intermediate or elevated sweat chloride concentrations. Most of these patients may have no evidence of respiratory tract or pancreatic abnormalities.

Summary

The diagnostic criteria and the tools to establish the diagnosis described above, will not cover every clinical situation. There will continue to be a clinical dilemma in a significant

number of cases in whom the diagnosis of CF is suggested by clinical presentation. Clinical judgment remains a central component of the assessment and monitoring of patients who lack conclusive evidence of CFTR dysfunction. A subset of these patients will require close clinical follow-up along and laboratory re-evaluation.

Hereditary pancreatitis

Familial pancreatitis describes the occurrence of this complication amongst multiple family members. In such situations, the pancreatitis could be due to a variety of known and unknown etiologies. Hereditary pancreatitis (HP) on the other hand, is characterized by acute, recurrent pancreatitis or chronic pancreatitis among individuals with a known disease causing mutation inherited in an autosomal dominant fashion. In patients with HP, attacks of acute pancreatitis generally begin in childhood but the onset of symptoms range from infancy to middle age. Acute attacks vary considerably in intensity, from mild abdominal discomfort to severe, potentially lethal attacks complicated by hemorrhagic necrosis, splenic vein thrombosis, and pseudocyst formation. Chronic pancreatitis, which usually follows recurrent, acute pancreatitis is no different, in term of symptoms to other causes of chronic pancreatitis. Patients frequently suffer from persistent, unrelenting pain, calcification within the parenchyma and ducts, fibrosis and ductal damage due to scarring and obstruction. After many years of chronic pancreatitis, maldigestion due to pancreatic insufficiency and diabetes mellitus will occur in a significant percentage of cases. Patients with chronic pancreatitis due to HP are susceptible to pancreatic cancer.

Hereditary pancreatitis gene

In 1996, Whitcomb et al identified the hereditary pancreatitis gene through mutation analysis of candidate genes within a specific locus of chromosome 7. A point mutation was identified in the cationic trypsinogen gene, which resulted in an arginine to histidine substitution in amino acid 117 (R117H) of the protein product. This particular mutation, which was identified in three family members with HP provided an initial clue to the molecular basis of this condition. Subsequently, this mutation was observed in DNA from all individuals in five kindreds that were being investigated.

Other mutations in the cationic trypsinogen gene have been identified in different kindred. This includes a point mutation in exon 2 (N29I), which has been identified in families from Europe, the United States and Japan. Additional mutations in the cationic trypsinogen gene that are associated with pancreatitis, include point mutations (K23R, A16V) or deletions (-28delTCC). It is expected that the number of pancreatitis associated mutations in the cationic trypsinogen gene will grow. It will be important to determine whether each molecular change actually confers pancreatitis.

Screening for cationic trypsinogen gene mutations

New techniques have emerged which offer the potential of screening for multiple mutations. This includes single-strand confirmation polymorphism (SSCP), denaturing grading gel electrophoresis (DDGE), heteroduplex analysis, and chemical mismatch cleavage. Other methods include enzyme mutation detection (EMD) and cleavase fragment length polymorphism (CFLP). Several commercial kits are now available for mutation scanning in the research setting.

How mutations in the cationic trypsinogen gene cause pancreatitis

Unlike cystic fibrosis, there appear to be only a limited number of specific mutations in the cationic trypsinogen gene. This is to be expected because unlike CF, HP is an autosomal dominant disorder and dominant mutations usually influence a critical regulatory role of the gene product. In the case of hereditary pancreatitis there appears to be a loss of inhibitory regulation. For example, the R117H mutation appears to make trypsinogen and trypsin more resistant to inactivation by the formation of pancreatic secretory trypsin inhibitor complexes. When there is a loss of ability of the pancreatic trypsin inhibitor to inhibit trypsinogen, trypsin auto-activation will occur, leading to pancreatitis. Other mutations may cause susceptibility to pancreatitis by different mechanisms.

Genetic penetrance

As in many other genetic conditions, caused by autosomal dominant inheritance, non-penetrance occurs in HP. Current data suggest that approximately 20% of individuals who carry a HP gene never develop pancreatitis. In individual cases, postmortem analysis has been performed and samples of the pancreas examined. No histologic evidence of acute or chronic pancreatitis was observed. Whether environmental or other genetic factors determine penetrance, the severity of disease or the age of onset of pancreatitis remains to be determined. Twin studies have been instituted in large kindreds to attempt to answer these questions.

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Screening for hemochromatosis and Wilson Disease

Bruce Bacon, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will gain an understanding of:

1. The value of screening for certain inherited liver diseases;
2. The use of genetic testing for evaluation of hemochromatosis;
3. The use of family screening for inherited liver diseases in children.

Screening for Hemochromatosis and Wilson Disease

Bruce R. Bacon, MD

Introduction

The issue of genetic screening for hereditary hemochromatosis (HH) and Wilson disease (WD) should be divided into a) screening for the disease either phenotypically or by genetic testing in patients with liver disease, b) family screening once a proband has been identified, and c) general population screening. Factors which affect all three of these types of genetic screening include a) availability of a reliable, relatively inexpensive screening test, b) the prevalence of the disease, and c) the presence of adequate therapy for the disease once the diagnosis has been made. Thus, for both disorders, the issues regarding the three types of genetic screening are quite different. For example, HH is common (1 in 200), a reliable genetic test is available, and treatment is effective, simple, and inexpensive. Conversely, WD is rare (1 in 30,000), and genetic diagnosis is complicated with over 60 mutations having been identified; however, effective treatment is available.

In this review, I will discuss these three types of genetic screening for these two inherited metabolic liver diseases and arrive at some recommendations for how we can use the available information in our practices. Understandably, the majority of information presented here will relate to HH, since the opportunities for screening are greatest in HH.

Hereditary hemochromatosis

Genetic defect

In August of 1996, the gene for HH was identified.[1] It was first called HLA-H and then renamed HFE. A single mutation in HFE where a cysteine is replaced by a tyrosine at amino acid position 282 (C282Y) was found homozygous in 83% (148 out of 178) of patients who had typical phenotypic HH. A second mutation where a histidine is replaced by an aspartate at amino acid position 63 (H63D) was also identified. The significance of this second mutation is still being determined. Between 15% and 20% of individuals are heterozygotes for H63D and it is clear that the same degree of iron loading does not occur with this mutation either in the heterozygous state or the homozygous state, when compared to individuals who are homozygous for C282Y.[2, 3] Numerous additional studies followed the initial report by Feder, et al., and it appears that about 85% to 90% of typical hemochromatosis patients are homozygous for C282Y. Another 3% to 5% of HH patients are compound heterozygotes with one allele containing the C282Y mutation and the other allele with the H63D mutation.

In addition to the obvious significance of this discovery in improving diagnosis and for screening, the discovery of HFE has opened the way for several studies which have begun to elucidate the pathophysiological mechanisms which are operative in normal iron absorption and in the dysregulated iron absorption that is found in hemochromatosis.[4] The creation of a HFE knockout mouse, which results in a phenotype similar to humans with HH (increased transferrin saturation, increased hepatic iron concentration in a parenchymal distribution), confirms that HFE is the gene responsible for hemochromatosis.[5] Immunohistochemical staining has shown that HFE is found in the crypt cells of the small intestine associated with b2-microglobulin and transferrin receptor. The C282Y mutated protein product of HFE inhibits transferrin receptor cycling leading to a “relative” iron deficiency in duodenal crypt cells. In turn, this results in an increase in production of the recently described divalent metal iron transporter-1 (DMT-1, also called DCT-1, and nRAMP-2) which is responsible for iron transport in the villus cells of the small intestine. Upregulation of DMT-1 has been confirmed in the HFE knockout mouse providing supportive evidence for this pathophysiological mechanism of increased iron absorption in HH.[6] Additional studies confirming these findings in humans with C282Y homozygous HH will be necessary.

Genetic testing in suspected HH patients

Hereditary hemochromatosis may be suspected if a patient presents with symptoms or physical findings that are suggestive of HH, if they have abnormal liver enzymes on screening blood tests, if they are known to have a family history of HH, or if they are found to have abnormal iron studies on routine screening chemistry panels. In these situations, if repeat fasting transferrin saturation values are elevated (> 50%) and/or if ferritin values are increased, then it is perfectly reasonable to proceed with genetic testing looking for the C282Y and the H63D mutations in HFE. If the individual has an elevated transferrin saturation, and is a C282Y homozygote, or a compound heterozygote (C282Y/H63D) then a liver biopsy can be avoided if the patient is under the age of 40 years and the serum aminotransferases are normal.[2] Using these criteria, we would not have missed any significant fibrosis had liver biopsy not been performed.[2] A large French study has come to the same conclusions except they also include a requirement for a serum ferritin being < 1,000 ng/mL.

If the transferrin saturation is elevated, but the genetic test is negative, then liver biopsy is necessary to determine the degree of iron loading. Occasionally, the question of a non-expressing C282Y homozygote arises. This is the situation where an individual is homozygous for C282Y but has normal iron stores. The true frequency of this abnormality is unknown and will only be identified after detailed population studies have been completed. Recent studies have shown new mutations in HFE that may be responsible for the iron loading that had been found in several patients with typical hemochromatosis who

lacked either the C282Y mutation or the H63D mutation. This area is still in its infancy.

Genetic screening in liver disease patients

Abnormalities in serum iron studies are common in patients with a variety of liver diseases. These abnormal iron studies are generally seen in patients with hepatocellular liver diseases, rather than in cholestatic liver diseases. Approximately 50% of patients with alcoholic liver disease, nonalcoholic steatohepatitis, and chronic viral hepatitis have serum abnormalities of iron metabolism.[4] These usually are an increased ferritin level, but occasionally patients can have an elevated transferrin saturation as well. Hepatic iron concentration is typically normal or slightly increased, but not to the level seen in HH. Genetic screening for the mutations found in HFE has now been applied to several groups of patients with alcoholic liver disease, nonalcoholic steatohepatitis, chronic hepatitis C, and porphyria cutanea tarda.

Grove, et al., examined HFE mutations in patients with alcoholic liver disease.[7] No increase in the prevalence of C282Y or H63D was found when compared to a control population, and there was no relationship between HFE mutations and hepatic iron levels in patients with alcoholic liver disease. Thus, despite many patients with alcoholic liver disease having abnormalities in serum iron studies, and some having an elevated hepatic iron concentration, it appears that mechanisms other than mutations in HFE are responsible.

In patients with chronic hepatitis C, the relationship between hepatic iron concentration and response to treatment with interferon has been known for several years.[8] Numerous studies have shown that patients who fail to respond to interferon monotherapy have a higher hepatic iron concentration than those patients who respond.[8, 9] This has led to the use of therapeutic phlebotomy to deplete iron stores prior to treatment with interferon; this treatment has typically led to a reduction in serum ALT levels without any significant change in HCV RNA levels. Recent studies in which HFE mutation analysis has been performed in patients with chronic hepatitis C have shown no difference in the prevalence of C282Y or H63D mutations in chronic hepatitis C patients when compared to a control population.[10, 11] However, a strong correlation between the presence of HFE mutations and abnormalities of iron metabolism, with an increase in serum ferritin level and an increase in hepatic iron concentration has been shown in those patients with at least one allele carrying the C282Y mutation. Further, in those patients with chronic hepatitis C who had C282Y heterozygosity and an increase in hepatic iron concentration, the fibrosis score was increased, suggesting a synergistic role for mild increases in hepatic iron in chronic hepatitis C causing progressive disease.[10] This provides further evidence in support of reducing iron levels in patients with chronic hepatitis C.

In nonalcoholic steatohepatitis (NASH) at least two studies have shown a high prevalence of the C282Y mutation (either heterozygotes or homozygotes) in patients with NASH.[12, 13] One study has shown an increase in fibrosis in those NASH patients with the C282Y mutation. Other studies have failed to show any increase in disease severity due to coexistent iron in NASH patients followed for up to 20 years.[14] It is still unclear as to whether the increased frequency of abnormalities in iron metabolism in these NASH patients represents a “referral bias” or if there truly is a definitive association between the presence of C282Y and nonalcoholic steatohepatitis. If this observation holds true, a role for phlebotomy therapy and iron reduction may be apparent as at least one form of treatment for patients with NASH. Since therapeutic phlebotomy is simple, inexpensive and safe, this form of therapy should be prospectively analyzed in a large group of patients with NASH.

Finally, in porphyria cutanea tarda (PCT), the relationship between abnormalities of iron metabolism and disease progression has been known for many years.[4] Recently a strong association with chronic hepatitis C has been identified in PCT patients.[15] It appears that this has geographic variation with the prevalence of hepatitis C positivity in PCT patients being much higher in Italy than in northern Europe or in the United States.[4] Relative to HFE mutations in PCT, several studies have shown that approximately 30% of PCT patients are either homozygous or heterozygous for C282Y, and iron reduction by therapeutic phlebotomy has been a mainstay for treatment of PCT for many years.[4]

Thus, the role of genetic screening for mutations in HFE seems quite valuable in patients with chronic hepatitis C, nonalcoholic steatohepatitis, and porphyria cutanea tarda, but it is less likely to be important in patients with alcoholic liver disease.

Family screening for HH

Once a HH proband has been identified, family screening is necessary and it is recommended in all first degree relatives. In the past, HLA-typing was recommended as a surrogate genetic test; with the advent of HFE mutation analysis, HLA-typing is no longer indicated. Both the C282Y and H63D mutations should be analyzed. In young probands with children, it is useful to perform HFE mutation analysis in the spouse in order to accurately predict the genotype in the children.[16] If the spouse has either mutation, then the children will also need to undergo HFE mutation analysis. Since C282Y and H63D are such common mutations, occurring in about 35% of individuals in one combination or another, there is approximately a one-third chance that the spouse will have a mutation in HFE.[2, 16] If C282Y homozygosity or compound heterozygosity (C282Y/H63D) is found in adult relatives of the proband, then serum iron studies should be requested. If transferrin saturation and/or ferritin levels are increased, then consideration for therapeutic phlebotomy should be entertained. In this situation, as long as liver tests (ALT, AST) are normal and ferritin is < 1000 ng/mL, liver biopsy is probably not necessary.[2]

Population screening for HH

In many respects, population screening for HH is a “no brainer.” HH has a number of features that fulfill criteria established by the World Health Organization for population screening. Hereditary hemochromatosis a) has a recognized latent or early asymptomatic stage, b) the natural history of the condition including its development from latent to declared disease is

adequately understood, c) there is a reliable screening test available, d) the test is acceptable to the population, e) there is an accepted policy on whom to treat, and f) screening has been shown to be cost-effective.[17]

Unfortunately, problems with case definition, phenotypic and genotypic definition, and which screening methods should be used must be clarified and further refined. Another concern relates to the issue that when a patient has been identified with a genetic disease, there may be a risk for losing health insurance, employment, or stigmatization from being labeled with a disease.[18] With the expected completion of the human genome project over the next several years, genetic diagnoses will become increasingly common and our state and national legislators must create appropriate legislation to prevent this type of genetic discrimination. Another important question relating to population screening is which members of the population should be tested and at what age. Hemochromatosis is a disorder of Northern European origin with the highest prevalence of disease having been reported from Ireland, Germany, Portugal, and western France.[4, 17] Despite genetic admixture in individuals of African or Asian descent, it has been extremely rare to find a non-Caucasian patient with a C282Y mutation. Thus, based on the available data, it is reasonable that hemochromatosis screening should be limited to Caucasian populations. When screening for iron overload (as opposed to screening for HFE-linked hemochromatosis), then extension to other racial groups should be considered. Since it is rare that HH causes any morbidity prior to age 20 years, screening could be limited to adults. Rather than every member of the population being evaluated, a more likely scenario is to increase patient and physician awareness and to screen all adults at one time when they visit a primary care physician.[17] Obviously, in America, with over 40 million uninsured citizens, this will not identify all those at risk but it may provide a start. The National Institutes of Health is currently supporting a large screening effort, whereby approximately 120,000 individuals in five or six sites across North America will be screened. This will provide valuable insight into how screening programs should be developed from the standpoint of a national public health policy.

Wilson Disease

Genetic defect

Wilson disease (WD) is a genetic disorder in which copper accumulates in the liver and brain in excess of normal metabolic needs. The defect in copper homeostasis in WD that leads to copper accumulation is a reduction in the biliary excretion of copper.[19] The Wilson disease gene was identified in 1993 and the gene has been designated ATP7B. The ATP7B gene is found on chromosome 13 and the predicted amino acid composition of ATP7B indicates that the protein resembles other adenosine triphosphate (ATP)-dependent metal ion transporters that have been highly conserved through evolution. ATP7B resides mainly in the trans-Golgi complex and is involved in copper transport into vesicles bound for lysosomes and the bile canaliculus; in addition, copper is transported into the secretory protein biosynthetic pathway for incorporation into ceruloplasmin. Unfortunately, there are a large number (>60) of disease-specific mutations and polymorphysims of the gene. The majority of the mutations thus far identified are point mutations that result in amino acid substitutions. However, deletions, insertions, and missense and splice site mutations have also been reported. The most frequently observed point mutations results in a change of histidine at amino acid position 1069 to glutamine (His 1069 ® Glu, H1069Q) and is present in nearly 30% of WD patients of European descent. In one Austrian population, the frequency of this mutation was reported to be as high as 65% in a group of WD patients.[20] The identification of the gene for WD has enabled molecular genetic diagnosis of the disorder. However, given the numerous disease-specific mutations of the gene now described and the observation that the most common mutation is present in only about 30% of most populations, direct analysis for the presence of disease-specific mutations is very difficult.

Screening for WD in patients with liver disease

All young patients (< 40 years) with new onset liver disease should be evaluated for WD at some point by way of a serum ceruloplasmin level. It would not be appropriate to do genetic testing in these individuals. If a ceruloplasmin level is reduced and the clinical setting is appropriate, then additional studies aimed at confirming the diagnosis of Wilson disease should be completed. These would include slit lamp examination for Kayser-Fleischer rings, liver biopsy with hepatic copper determination, and 24 hour urine collection for copper excretion. These studies should also be performed if clinical suspicion of WD is high, even if the ceruloplasmin level is normal. Unfortunately, genetic testing is of limited value in this situation given the large number of mutations that have been identified.

Family screening for WD

Once a proband with Wilson disease has been identified, it is possible to perform genetic testing in siblings to determine their susceptibility to the disease. Unfortunately, this is only available in a few research laboratories worldwide and must be done along with standard clinical and biochemical testing.[19]

General population screening

Generalized population screening for Wilson disease is impractical given the low prevalence of the disease and the lack of a readily available, reliable genetic test.

Summary

In summary, population screening for hemochromatosis may become a reality at some time in the future. Mutation analysis for C282Y and H63D is reasonable in patients with a variety of liver diseases to determine if there is a component of that individual's disease that could be related to mild increases in iron as seen in C282Y heterozygosity. There is no role for generalized population screening in Wilson disease. Family screening is appropriate for both diseases and screening for Wilson disease should be performed in all patients who present with abnormalities in liver function.

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Notes

Screening for viral hepatitis – Pediatric epidemiology

Maureen Jonas, MD

LEARNING OBJECTIVES:

At the conclusion of this session, participants will understand that:

1. Rational viral hepatitis screening policies and recommendations require an understanding of the epidemiologic features of these infections;

2. Epidemiologic features of viral hepatitis are different in pediatric than adult populations, and vary by geographical region, socioeconomic and living conditions, and risk factor history;

3. Epidemiologic patterns of viral hepatitis infections form the basis for control and eradication strategies.

Screening for Viral Hepatitis—Pediatric Epidemiology

Maureen Jonas, MD

Introduction

Although the prevalence of infectious hepatitis is higher in adults, there are specific issues regarding epidemiology, modes of transmission and immunization strategies that are unique to the pediatric population. These issues and their impact on the current approach to identification, prophylaxis and elimination of these diseases will be reviewed.

Hepatitis A

Although in older children and adolescents the illness caused by HAV is more like that seen in adults — with fever, abdominal pain, anorexia, and jaundice — symptoms of HAV infection in young children may be minimal, or resemble those of viral gastroenteritis. These unrecognized HAV infections in young children represent an ongoing reservoir in a community or daycare setting, causing secondary cases and perpetuation of outbreaks.

Table 1 describes the worldwide endemicity patterns of HAV. In underdeveloped countries, with inadequate hygiene and sanitation, most children are infected in the first few years of life. In developed countries, infection is uncommon early in life and serologic evidence of infection gradually increases with age. The importance of this pattern is that lack of infection in early life leads to a population of susceptible adults in which the clinical illness is more severe. Infants and children may shed HAV for longer periods than adults, up to several months after the onset of clinical illness, representing a long-lasting reservoir for transmission. There is greater risk of transmission under conditions of close personal contact, such as in households, day care centers, military camps, or residential institutions. Although maternal-neonatal transmission has been reported, it is rare. During community outbreaks in the developed countries, the highest rates of infection occur in children, adolescents and young adults. In the United States, rates of infection vary among ethnic groups and by geographical distribution. This is probably due to socioeconomic differences and degree of crowding in living conditions.

Table 1

Endemicity of Hepatitis A Virus Infection

ANTI-HAV PREVALENCE		
Low	Intermediate	High
North America	Russia	Central and South America
Western Europe Australia and New Zealand	Eastern Europe	Asia Africa

In the United States, there were approximately 30,000 reported cases of HAV infection in 1997. This figure is believed to underestimate the true incidence because of non-reporting of subclinical infections. In the general population, the reported incidence of hepatitis A is highest among children 5-14 years of age, with approximately one third of reported cases involving those younger than 15 years of age. Children play an important role in HAV transmission and serve as a source of infection for others because they often have asymptomatic or unrecognized infections. In a 1997 study of adults without an identified source of infection, 52% of their households included a child younger than 6 years of age; the presence of a young child was associated with HAV transmission within the household. Approximately 11-16% of reported cases occur among children or employees in day care centers or among their contacts. Earlier data from 1994 indicated that 10% of the cases reported to the CDC occurred in day care centers for non-toilet-trained children. In the United States, hepatitis A cases in elementary or secondary schools usually reflect disease acquisition in the community. Child to child transmission within the school setting is uncommon. If multiple cases occur among children in a school, a common source of infection is usually implicated. Four to 6% of cases in the United States occur among international travelers. Children account for one-third of these infections, with Mexico being the most common travel destination.

In developed countries, strategies to control and perhaps even eradicate HAV infection are less based on standard public health measures and more centered on methods to elicit individual and population immunity. Prevention of HAV infection can be conferred passively with immune serum globulin or actively by vaccination. Two HAV vaccines, HAVRIX® (SmithKline Beecham Biologicals) and VAQTA® (Merck & Company, Inc.) have been licensed. Polyvalent vaccine made from one strain provides protection against all. Immune serum globulin is not routinely indicated when a single case occurs in an elementary or secondary school and the source of infection is outside the school, because of the low frequency of child-to-child transmission in that setting.

Children are a primary focus of immunization strategies because of high disease incidence and their critical role in HAV transmission. Recently, the Immunization Practices Advisory Committee of the Centers for Disease Control has published revised recommendations for pediatric HAV immunization. These are summarized in Table 2, which lists states in the U.S. where children should be routinely immunized and those in which vaccination for children should be considered because of a higher than average disease incidence. Targeting of age groups recommended for vaccination should take into consideration community disease patterns. In communities with high

prevalence, such as American Indian reservations or Alaskan Native villages, routine vaccination of children should begin at 2 years of age, and preschool children should receive "catch up" vaccination. Pre-vaccination serologic testing of this group is not necessary. Immunization of older children is also recommended. Pre-vaccination serologic testing may be considered for older adolescents and adults who were either born or lived for extensive periods in geographic areas that have a high endemicity of HAV, or are part of a population at increased risk (Native American or intravenous drug user) for this disease.

Table 2

States Where Routine Childhood HAVVaccination is Recommended or Should be Considered

Recommended	Considered
(Based on average reported incidence of > 20 cases per 100,000 population OR > twice national average from 1987-1997)	(Based on average reported incidence of > 10 cases per 100,000 population OR > national average but < twice national average from 1987-1997)
ARIZONA	MISSOURI
ALASKA	TEXAS
OREGON	COLORADO
NEW MEXICO	ARKANSAS
UTAH	MONTANA
WASHINGTON	WYOMING
OKLAHOMA	
SOUTH DAKOTA	
IDAHO	
NEVADA	
CALIFORNIA	

The similarities in epidemiology of hepatitis A and poliomyelitis indicate that a generalized reduction in disease incidence, and perhaps even eradication, can be achieved by immunizing persons in age groups that have the highest rates of infection, who serve as the reservoir. It has been observed that routine vaccination of children living in communities with the highest rates of disease has been effective in interrupting ongoing outbreaks and preventing subsequent outbreaks in these communities. However, to achieve a sustained reduction in the national incidence of hepatitis A, widespread routine vaccination of children would be required. Although a vaccine for children under 2 years is not yet available, routine vaccination of older children would help to achieve effective control and prevention of this disease.

Recommendations

Hepatitis A is an acute, self-limited disease with good outcome in the majority of children. Screening is not usually recommended in clinical practice, and testing for prior infection is not usually necessary when vaccination is being considered. Screening is done in the context of epidemiologic surveys, in order to delineate target populations for immunization and eventual HAV elimination.

Hepatitis B

The World Health Organization estimates that the carrier rate of hepatitis B virus (HBV) will soon reach 400 million. In the United States, the estimated number of new cases of HBV infection is 200,000 to 300,000 per year, and an additional one million people are chronic carriers. The mortality rate attributed to HBV infection in this country is 5,000 per year, with 350 deaths due to fulminant hepatitis, 4,000 due to end stage liver disease and 800-1,000 from hepatocellular carcinoma. Although the epidemiology of HBV infection in children plays an important part in the patterns of illness, the mortality in children is minimal.

The modes of transmission and the epidemiology of HBV infection vary in different parts of the world and are related to the endemicity in the population. Table 3 lists the prevalence of HBV infection in different parts of the world. Densely populated areas in Africa and Asia, as well as some isolated groups, such as Eskimos in Alaskan villages, have been identified as high prevalence groups. Although the United States, Western Europe and Australia are considered low prevalence regions, there are subgroups within these populations with much higher carrier rates, such as parenteral drug users, homosexual men, and immigrants from endemic areas. In high-endemicity areas, infection occurs early in life because of a high incidence of vertical (maternal-neonatal) and horizontal (child-to-child) transmission.

Table 3

Worldwide Endemicity and Prevalence of Hepatitis B Virus

Low (<2%)	Intermediate (2-7%)	High (≥8%)
North America (except Alaska)	Brazil	Southeast Asia
Central and South America (except Brazil)	Russia	China
Western Europe	Eastern Europe	Africa
Australia		Eskimos in Alaska

In the United States, children represent only a small proportion of HBV infections. Children 11-19 years of age comprise approximately 10% of new cases, and those younger than 10 years of age, 8% of new cases. Risk factors for HBV acquisition in children and adolescents include perinatal exposure, horizontal transmission, and parenteral exposure to blood or blood products. Adoptees from some countries, such as China, India, Russia and Romania, have a particularly high prevalence of HBV infection. In adolescents, risk for HBV infection is associated with sexual activity, i.e. numerous sexual partners and other sexually transmitted diseases, and parenteral drug use.

Horizontal transmission is unique because it occurs almost exclusively during childhood; it is defined as transmission that excludes perinatal, parenteral and sexual infection. Horizontal transmission seems to be the primary mode of HBV acquisition in parts of the world with moderately high endemicity, including sub-Saharan Africa, South Africa, parts of Southeast Asia, and the Middle East. In the United States, there is a relatively high

risk among children of Southeast Asian descent. In the case of horizontal HBV transmission, children are born to hepatitis B surface antigen (HBsAg) negative mothers but subsequently go on to develop HBV infection in the first years of life. The specific mechanisms of this process are not clear, although transmission via contact with infectious sera from dermatologic lesions or oral sharing of contaminated articles has been suggested.

The blood of infected individuals contains high concentrations of HBV; moderate concentrations are found in semen, vaginal fluid and saliva. Although low levels of virus have been found in breast milk, studies from Taiwan and England have suggested that breast-fed infants of HBsAg positive women are not at significant risk of infection.

There is no evidence of transmission of HBV in day care centers. Although transmission of HBV has been documented among mentally retarded individuals in institutional settings, and rarely in classrooms with normal individuals, the magnitude of the risk appears to be low, and is probably related to poor hygienic practices or aggressive behavior such as biting and scratching. Younger children, however, may manifest only vague non-specific symptoms, and the illness may be unrecognized.

HCC associated with childhood HBV infection has been described, and cases in children as young as 8 months old have been reported. Periodic measurement of serum alpha-fetoprotein levels and hepatic ultrasounds are recommended as surveillance for early HCC in chronically infected adults. Currently, there are no widely accepted guidelines for prospective monitoring for development of HCC in children with chronic HBV infection.

It is hoped that an aggressive HBV vaccination policy during infancy and early childhood will markedly decrease and possibly eradicate chronic HBV infection and HBV-associated hepatocellular carcinoma in the United States. In Taiwan, where perinatal transmission was the predominant mode of HBV acquisition, interruption of this cycle by universal infant immunization has drastically reduced the prevalence of childhood HBV infection. This in turn has led to a marked decrease in hepatocellular carcinoma in this population. Recently, HBV vaccine has been designated the first “anti-cancer vaccine” by the Food and Drug Administration.

Recommendations

1. Testing of all pregnant women for HBsAg has been recommended for more than 10 years.
2. Children born to HBsAg positive mothers should undergo screening if they have not received immunoprophylaxis.
3. Children born to HBsAg positive mothers who did receive immunoprophylaxis should be tested for HBsAg and Ab to confirm protection or document in utero transmission.
4. Children who are either immigrants or adoptees from areas with high endemicity should be screened for HBsAg.
5. Children who live in sub-populations with high endemicity should be screened for HBsAg.
6. Screening is not recommended for adolescents prior to immunization.

Hepatitis C

It has been estimated that 3% (170 million) of the world’s population has been infected with hepatitis C virus (HCV). It is the most common cause of non-A, non-B hepatitis. Prevalence rates by geographic region are listed in Table 4. In the United States, there are about 2.7 million individuals chronically infected with HCV and approximately 28,000 new infections occur each year. Young adults are at highest risk for HCV acquisition. In the United States, there is wide variation in prevalence among different subgroups. In those with repeated percutaneous exposures, such as injecting drug users, the prevalence is 60 to 90%, while in health care workers it is 1%. The HCV antibody seroprevalence is 0.2% in children younger than 12 years, and 0.4% in those 12 to 19 years of age. Children at risk for HCV infection, along with prevalence rates, are listed in Table 5.

Until recently, blood or blood products (such as clotting factors, plasma and intravenous immunoglobulin) have been the major mode of transmission of HCV to children. As has been

Table 4

Childhood HCV Prevalence Rates Around the World

Country	Prevalence
Japan, Taiwan, Egypt	0%
Italy	0.4%
Saudi Arabia	0.9%
Cameroon	14.5%
United States	0.1%-0.4%

Table 5

Risk Factors for HCV in Children and Associated Prevalence Rates

Risk Factor	Prevalence
Multiple ongoing blood transfusion:	
Hemophiliacs (treated with unheated or dry heat-treated factor VIII or IX concentrates)	Up to 95%
Thalassemics	61-63%
Hemodialysis	15-20%
History of moderate (but not ongoing) transfusion exposure from:	
Childhood leukemia	4-9%
ECMO*	8%
Cardiac surgery	4-5%

*extracorporeal membrane oxygenation

described for adults, the risk of acquiring HCV increased with the number of units of blood or blood products received. Transmission to children has also occurred from transplanted organs or tissues.

Children treated for leukemia typically receive many blood transfusions, and those treated prior to 1990 have a very high rate of HCV infection. Children with thalassemia requiring chronic transfusions have a very high prevalence of HCV infection. Many individuals with hemophilia contracted HCV during childhood, before the availability of heat-inactivated and recombinant clotting factors. The frequency of viral persistence is lower than that reported in adults who were infected via transfusion. The reason for this relatively high spontaneous clearance rate is not clear.

Screening of blood for antibody to HCV, the use of recombinant and heat inactivated clotting factors, and the addition of virus-inactivating physicochemical processes in the production of immunoglobulin have virtually eliminated HCV transmission from these sources. Since HCV has been almost eliminated from the blood supply, perinatal transmission is becoming the primary mode of transmission to children. The incidence of new infections per year through this mechanism is not known, but can be approximated by the prevalence of HCV infection in women of childbearing age (0.13 to 1.29%), the birth rate, and the risk of transmission with each pregnancy (5 to 10%).

Selected studies of perinatal transmission are summarized in Table 7. Several studies have demonstrated a 5-6% rate of perinatal transmission from anti-HCV seropositive women. The transmission rate increases to 10 to 11% for women who have HCV RNA in serum at the time of delivery. A rate of as high as 33% was reported in one Italian study, but this has not been consistently observed. The likelihood of HCV transmission increases with higher levels of maternal HCV viremia and maternal coinfection with human immunodeficiency virus (HIV) increases the rate of perinatal HCV transmission. Whether vaginal delivery is associated with a greater risk relative to cesarean delivery is not clear. Transmission frequencies of 4% after vaginal delivery and 6% after cesarean delivery were reported in one cohort, whereas no cases of perinatal transmission occurred after cesarean delivery in another series. Maternal HCV infection is not currently considered an indication for cesarean delivery.

HCV transmission has been documented in women who have acute infection during the last trimester of pregnancy. The possibility of in utero transmission has been suggested by the detection of viremia in six infants on the day of birth. In some instances, viremia in the neonates is transient and not associated with the development of liver disease.

There have been conflicting results regarding the role of breast-feeding in the risk of HCV transmission to infants. As shown in Table 7, when breast-feeding was reported in perinatal transmission studies, the proportion of HCV infected children who were breast-fed ranged from 3% to 46%. The significance of this wide range is uncertain without knowing the frequency of breast-feeding in the study populations. Differences in the

Table 7

Summary of Selected Studies of Perinatal HCV Transmission

Author and Year	Pregnancies at Risk (N)	% Transmission (HCV-RNA+)	Risk Factors for Vertical Transmission
Novati, 1992	5 RNA + and HIV +	80	
Reinus, 1992	23 pregnancies with 24 infants Ab or RNA+	0	
Wejstal, 1992	14 pregnancies 21 children Ab and RNA+	4.7	
Lam, 1993	66 Ab and RNA+	6 (Ab only)	
Marcellin, 1993	10 Ab and RNA+	0	
Roudot-Thoraval, 1993	18 Ab+	0	
Ohto, 1994	53 Ab+ 31 RNA+	5.6 20	HCV RNA titer $\geq 10^6$/ml
Lin, 1995	15 Ab and RNA+	0	
Manzini, 1995	45 Ab+ 27/45 HIV- 18/45 HIV+	2.2	
Paccagnini, 1995	70 Ab+ (37/70 RNA+) 53/70 HIV+ 17/70 HIV-	20 23 12	Maternal HIV Vaginal delivery
Zanetti, 1995	116		Maternal HIV status
	94/116 HIV- 22/116 HIV+	0 36	
Zuccotti, 1995	21 RNA+ 13/21 HIV+ 8/21 HIV-	 30 25	
Sabatino, 1996	30 Ab+ 10/30 RNA+	10 33	HCV viremia during third trimester
Tovo, 1997	245 Ab+ 165/25 HIV+ 80/245 HIV-	11.4 15.1 3.7	Vaginal delivery
Giacchino, 1998	45 RNA+	8.9	ND
Granovsky, 1998	122 Ab or RNA+	5.7	HIV coinfection, High HIV load Vaginal delivery
Resti, 1998	403 Ab+ 275/403 RNA+	3.2	Maternal IVDA, post-transfusion vs. no risk factor

ND not done; NA not applicable; HIV human immunodeficiency virus; IVDA intravenous drug abuse

duration of breast-feeding were noted between uninfected and infected infants in a Japanese study. In a small series from Taiwan, none of 11 breast-fed infants had documented infection at one year of age. Two studies from Italy suggest a lack of additional risk from breast-feeding. In the first, none of 17 breast-fed infants born to HCV+, HIV- mothers was infected after follow-up of 12-27 months. In the second, the perinatal transmission of HCV in breast-fed infants was 7% compared to 4% in formula fed infants, but this difference was not statistically significant. None of 76 breast milk samples from 73 anti-HCV positive German women contained HCV RNA even though 62 were HCV RNA seropositive. Only one child had evidence of HCV infection detected one month after birth. Based on these limited data, there is currently no recommendation to avoid breast-feeding by HCV infected women.

Currently, there are no specific recommendations to decrease the frequency of perinatal transmission from HCV infected women. Neither post-exposure prophylaxis of the newborn with immune globulin or elective cesarean delivery appears to be effective in preventing HCV infection and are not recommended. However, if data from recently reported CDC studies are confirmed, avoidance of internal fetal monitoring and delivery prior to 6 hours of ruptured membranes may be recommended for HCV-infected women. In that case, routine screening to identify these women may be necessary.

Horizontal transmission of HCV is possible but probably uncommon. Two important issues regarding horizontal HCV transmission in the pediatric population are the frequency with which children are infected by affected family members and the frequency with which HCV-infected children transmit infection to others in the home or school. Studies of household transmission have been done in South America, Europe and Asia by screening contacts of HCV-infected individuals for anti-HCV. Some have also tested seropositive cases with PCR for HCV RNA and comparative genotype analysis. Prevalence of anti-HCV seropositivity in household contacts varied from 0-14.8% and non-sexual (non-spouse) seroprevalence rates were 0 to 6.5%. Higher rates were seen in the households of HCV-infected individuals with liver disease compared to those who were asymptomatic carriers and in households of those with risk factors vs. those without. In households of HCV-infected hemophiliacs, rates were particularly low.

There are few data regarding transmission of HCV from one child to another. A study from Spain examined 80 household contacts of 27 HCV-infected children. They had no other risk factors for infection. One sibling and no parent was infected. In an Italian study of 44 index children, no transmission to other children was demonstrated, although one parent was infected from a needlestick. It appears that horizontal transmission of HCV between children is rare, and there is no need to restrict school, day care or contact sports participation in HCV-infected children.

Recommendations

1. Routine screening of pregnant women is not recommended at this time. (However, recent data suggest that internal fetal monitoring and prolonged rupture of membranes may be risk factors for perinatal transmission; if these data are confirmed, this recommendation may change.)
2. Women who are pregnant or considering pregnancy and who have risk factors such as current or past intravenous drug use, blood transfusions prior to 1992, or unexplained ALT elevations, should be offered testing for anti-HCV. Those found to be anti-HCV positive should undergo PCR testing to confirm infection.
3. Infants born to mothers infected with HCV during pregnancy or at delivery should be tested after one year of age for anti-HCV; testing in the first few months of life may produce false positive results, since antibodies are placentally transferred from mother to infant. A positive test after 12-15 months of age is likely to correlate with true infection.
4. Screening is not recommended for household contacts who are not sexual partners or children of infected women.
5. Children treated with transfusion of blood or blood products prior to 1992 should be screened with testing for HCV antibody. However, those treated for leukemia may not develop anti-HCV, and PCR testing may be necessary.
6. Adolescents with a history of intravenous drug injection, sexually transmitted disease, or multiple sexual partners should be screened. It is not clear if adolescents with tattoos or body piercing are at risk for HCV infection.

Summary

Hepatitis A, B and C are international public health issues in which children play important epidemiologic roles. With respect to HAV, children often have subclinical infections and act as reservoirs for transmission to adults who have more severe disease. Improved sanitation has been clearly shown to decrease the incidence, but widespread routine vaccination of children could help to achieve effective control and prevention of HAV. Perinatal HBV transmission is critical in maintaining the endemicity of HBV in a population. Horizontal transmission of HBV occurs almost exclusively in childhood, although the mechanisms are not clear. Immunoprophylaxis of infants and children remains a critical strategy in preventing HBV transmission and its complications, such as cirrhosis and hepatocellular carcinoma. The goal of elimination of HBV infection has become a real possibility. Although the prevalence of HCV infection in the general pediatric population is low, there is a large population of infected women of childbearing age. Perinatal transmission has become the major route of infection for children, and interruption of this cycle will have a significant impact on new cases. Understanding the sources of viral hepatitis in children, and attempts to prevent new cases, will have broad implications for both child health and public health throughout the world.

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Notes

Inborn errors of fatty acid metabolism and liver disease

Piero Rinaldo, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will:

1. Develop an understanding of the clinical and biochemical manifestations of disorders of fatty acid transport and mitochondrial oxidation;

2. Recognize the potential link between recently described disorders and the occurrence of fulminant liver failure in children;

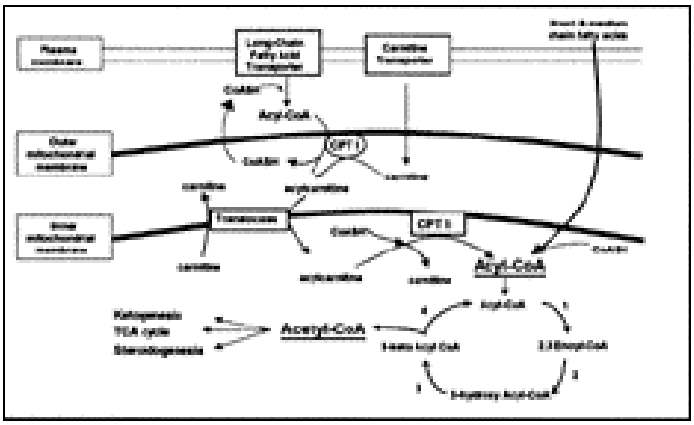
3. Learn a protocol of biochemical and in vitro studies to diagnose disorders of fatty acid metabolism.

Inborn Errors of Fatty Acid Metabolism and Liver Disease

Piero Rinaldo, MD, PhD

Fatty acid transport and mitochondrial oxidation

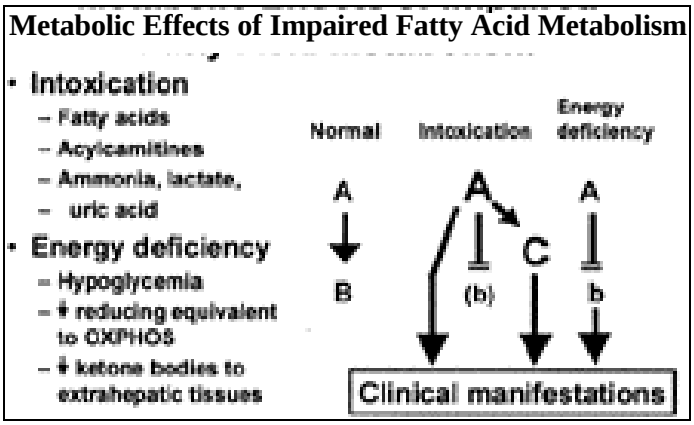
Fatty acid transport and mitochondrial oxidation (FATMO) is a metabolic pathway that plays a major role in energy production during fasting or periods of higher energy demands that lead to glycogen depletion.[1] When the body's glycogen stores are depleted, long-chain fatty acids are mobilized from adipose tissue and taken up by liver and muscle cells. While short- and medium-chain fatty acids (C4 to C12) diffuse freely across plasma and mitochondrial membranes, the transport of longer chain species depends at least in part upon active transport.[2] After activation to their respective coenzyme A esters by ATP-dependent acyl-CoA synthetases, fatty acids are transported into mitochondria by a series of three membrane bound enzymes which temporarily convert the acyl-CoA into an acyl-carnitine species before delivering a reconstituted fatty acyl-CoA into the inner mitochondrial space.



Fatty acids are oxidized to their final product, acetyl-CoA, in a cyclic fashion, which consists of four sequential reactions mediated by highly homologous enzymes. These enzymes are characterized by their affinity for substrates of decreasing chain length, hence the common nomenclature using prefixes such as very long-chain, long-chain, medium-chain, and short-chain. The catalytic activity of these enzymes are FAD-dependent acyl-CoA dehydrogenase, enoyl-CoA hydratase, NAD-dependent L-3-hydroxyacyl-CoA dehydrogenase, and ketoacyl-CoA thiolase, respectively. Each cycle of the pathway produces a molecule of acetyl-CoA and a fatty acid with two fewer carbons. Under physiological conditions, the latter re-enters the cycle until it is completely consumed. In the liver, acetyl-CoA constitutes the building block for the synthesis of ketone bodies (acetoacetic acid and D-3-hydroxy butyric acid), which serve as fuels for production of energy in other tissues when the supply of glucose is depleted.

Clinical manifestations of FATMO disorders

Inherited FATMO disorders represent a rapidly expanding class of metabolic diseases.[1,3] Pending a more accurate definition of the underlying molecular defects, at least 23 distinct disorders have been characterized to date, but this number is likely to increase. The pathogenic mechanisms underlying the clinical manifestations of FATMO disorders are similar to virtually all mitochondrial inborn errors of metabolism, originating from two basic mechanisms: intoxication and/or energy deficiency. In the intoxication type, substrate accumulation leads to direct toxicity (i.e., membrane disruption) and to inhibition of other enzymes. In the energy deficiency type, the accumulation of substrate could be relatively harmless but limited availability of the reaction product significantly impairs essential cell functions. In FATMO disorders both mechanisms clearly are involved: the accumulation of fatty acyl-CoA species, their secondary metabolites, and other by-products reflect the intoxication component; the shortage of acetyl-CoA and reducing equivalents impairs hepatic ketogenesis and the synthesis of ATP by the oxidative phosphorylation pathway and results in cellular energy depletion.



The major clinical manifestations associated with individual FATMO disorders are hypoketotic hypoglycemia, transient to fulminant liver disease, skeletal myopathy, dilated/hypertrophic cardiomyopathy, and sudden and unexpected death in early life. Signs and symptoms may vary greatly in severity and may appear at any age, from birth to adult life, and in variable combinations, frequently leading to life-threatening episodes of metabolic decompensation after a period of inadequate caloric intake and/or intercurrent illness. Despite the distinctive features of fasting intolerance and hypoketotic hypoglycemia, however, a prompt diagnosis of FATMO disorders is often delayed by the greater consideration given to some clinical entities of multiple and/or uncertain etiology, such as Reye syndrome, fulminant liver failure, and neonatal compromise

thought to be secondary to maternal liver complications of pregnancy.[4]

The biochemical genetics approach to disorders of fatty acid metabolism

In the event of an episode of metabolic decompensation, selected routine and specialized laboratory tests should be performed at the earliest possible opportunity.[5] Typically, but not always, they reveal hypoketotic (in urine) hypoglycemia, the signature finding that most often raises the diagnostic possibility of a FATMO disorder. Once a suspicion has been raised, it is necessary to rely on specialized biochemical investigations to formulate a tentative diagnosis of a particular defect.

Laboratory Investigations

Routine	Specialized
BLOOD	BLOOD
– Gases, Electrolytes	– Canitine
– Glucose	– Acylcarnitines
– Lactate, pyruvate	– Fatty acids
– Ammonia	– 3-OH Fatty acids
– LFTs, CPK	
URINE	URINE
– Ketones	– Organic acids
	– Acylglycines

The differential diagnosis of a specific FATMO disorder is an increasingly complex process that cannot be achieved by a single test, but requires the performance of multiple analyses and their integrated interpretation. Plasma and urine specimens should be obtained at the earliest possible stage of an acute episode as the presence of biochemical markers in plasma and urine may be intermittent. Unless a patient is evaluated at the height of an acute episode or in response to closely supervised provocative tests, a comprehensive diagnostic work-up could be completely uninformative. Furthermore, partial deviations from a "model" pattern are always possible, for the variable severity of a particular metabolic block and the role that environmental factors could play in individual patients.

A biochemical diagnosis always needs to be confirmed by enzymatic and/or molecular studies. Such verification is crucial as some patients present with metabolite profiles that may indicate a specific defect, but are not supported by normal or uninformative results of in vitro studies. Enzymatic and molecular studies are therefore a necessity, as provocative studies in vivo (for example, a fasting test) may be limited by age consideration and by the desire not to expose a patient to unnecessary risks.[1] A common approach is to perform a skin biopsy to establish a line of cultured fibroblasts to be utilized for a variety of enzymatic and functional assays. The advantage of any analytical approach that utilizes cultured cells is that the material can be grown in sufficient quantity to provide additional confirmatory enzyme and molecular studies without having to take additional patient samples.

In Vitro Studies

Analysis of cultured fibroblasts

– Metabolic flux studies

– Direct measurement of enzymes and transporters activity

– Complementation

Molecular studies

Recently described FATMO disorders and liver disease

Although our understanding of the biochemical and molecular basis of FATMO disorders has improved dramatically in recent years, clinically suspected cases often remain undiagnosed despite extensive laboratory investigations in vitro. Such patients are considered affected with an "unspecified" fatty acid oxidation disorder, a less than ideal but nevertheless appropriate clinical concept, as new discoveries often stem from the study of these atypical and valuable cases.

FATMO Disorders and Liver Failure

Three newly described FATMO disorders present with acute liver failure requiring in some cases liver transplantation (LT)

– LCFA transport defect (6 cases, 3 LT)

– Liver SCHADdeficiency (13 cases, 1 LT)*

– S/MCHAD deficiency (1 case, 1 LT)

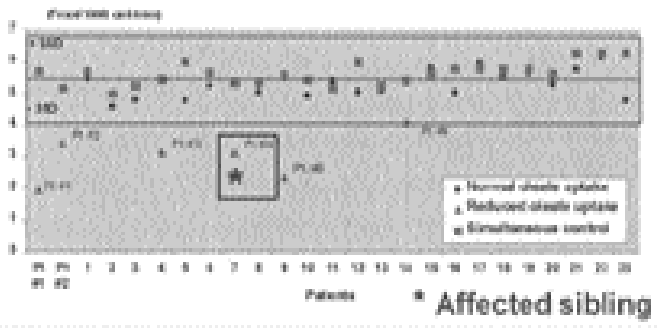
*(Michael J. Bennett, unpublished results)

Long-chain fatty acid transport deficiency

Two patients affected with a defect of long-chain fatty acid transport at the plasma membrane level have been reported.[6] The first patient had a history of recurrent acute liver failure and uninformative or non-specific findings of biochemical investigations. In some of the episodes, hyperammonemia and encephalopathy were also present, but skeletal or cardiac myopathy were never observed. Growth and development were normal and between episodes the patient was entirely well. At five years of age, however, he underwent orthotopic liver transplantation following a life-threatening episode which evolved into chronic hepatic insufficiency. The second patient presented with fulminant liver failure at four years of age and also underwent liver transplantation. Both patients are doing well several years post-transplant. In cultured skin fibroblasts, the oxidation rates of C14-C18 fatty acids were reduced with the severity of the defect correlating to the chain length of the substrate, but were normal in digitonin-permeabilized cells. The site of the defect was confirmed by reduced velocity of C14-C20 fatty acid uptake, while the uptake of 2-deoxy-D-glucose, carnitine, and palmitoyl-L-carnitine were normal.

A preliminary report of the investigation of 19 patients with unexplained liver failure showed a reduced oleate uptake in five additional patients (two siblings).[7] These data suggest that a defect of long-chain fatty acid transport could be a relatively common finding among patients presenting with either acute

Screening by Oleate Uptake Activity in Cultured Skin Fibroblasts



dysfunction or fulminant liver failure. All cell lines showed the ability to normalize oleate transport activity when fused with cell lines of normal and disease controls. The two index cases did not show correction of the uptake defect after complementation, providing additional evidence that they are affected with the same primary defect. However, any other combination lead to normalization of oleate uptake, suggesting the possible existence of multiple defects manifesting with impaired long-chain fatty acid uptake in vitro. Sequencing and immunoblotting experiments targeting several candidate genes have been uninformative to date (unpublished results).

Short/medium chain L-3-hydroxy acyl-CoA dehydrogenase deficiency

The proband was a 3-year-old Caucasian girl with previous normal growth and development. She presented with flu-like symptoms and low grade fever, for which she was given two doses of acetaminophen. Over a period of six hours, she became progressively unresponsive and was admitted to the hospital. Preliminary lab investigations revealed hypoglycemia (35 mg/dL) and hyperammonemia (145 U/L), elevated transaminases (AST 435 U/L, ALT 276 U/L), no ketonuria. A liver biopsy showed centrilobular necrosis and periportal steatosis. At the time of presentation, the most significant biochemical finding was a transient but marked elevation of plasma medium-chain (C6-C12) 3-hydroxy free fatty acids.[8] After a diagnosis of fulminant liver failure was made, she received a living-related (maternal) transplant within 48 hours. Although the post-operative course was complicated by renal tubular necrosis and she required tube feeding for one year, she gradually recovered and is doing well two years after transplant. Short and medium chain L-3-hydroxyacyl-CoA dehydrogenase (S/MCHAD) catalyzes the NAD-dependent oxidation of 3-hydroxyacyl-CoA for C4-C16 substrates. In this patient, mutations have been found in the conserved NAD-binding domain of the S/MCHAD gene.[9]

Short-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency

The first reported patient with SCHAD deficiency was an adolescent presenting with juvenile-onset recurrent myoglobinuria, ketonuria, hypoglycemia, encephalopathy and cardiomyopathy who died with acute metabolic illness. Using a C4 ketoacyl-CoA as substrate in the reverse reaction she was shown to be deficient in SCHAD activity in skeletal muscle, but not in cultured skin fibroblasts. Later, two unrelated patients who presented with recurrent episodes of ketotic hypoglycemia with 3-hydroxy dicarboxylic aciduria were identified with a defect of catalytic activity using the same assay that was expressed in skin fibroblasts. More recently, a number of patients presenting with either sudden unexpected death or severe liver disease were found to have a defect that seems to be a liver specific isoform (MJ Bennett, personal communication), with normal SCHAD activity in muscle and fibroblasts.[10] SCHAD deficiency in all of these patients was described enzymatically as no mutations have been found by sequencing the putative SCHAD gene.

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The enteric nervous system and GI motility

Carlo DiLorenzo, MD

LEARNING OBJECTIVES:

At the conclusion of this session, participants will be able to:

1. Recognize the role of the enteric nervous system in generating and coordinating gastrointestinal motility;

2. Understand the importance of bi-directional communication between central and enteric nervous system;

3. Understand how progress in understanding of the enteric nervous system is helping in clarifying the pathophysiology of common gastrointestinal diseases and in developing new treatment modalities for functional bowel disorders.

The Enteric Nervous System and GI Motility

Carlo Di Lorenzo, MD

The enteric nervous system

The enteric nervous system (ENS) is embedded within the walls of the digestive tract and consists of two interconnected plexuses, the myenteric (Auerbach's) and the submucous (Meissner's) plexus. Such layers of complex microcircuitry envelop yards of hollow organs and contain more neurotransmitters and neuromodulators than can be found anywhere else in the peripheral nervous system. Structurally (presence of enteric glial cells resembling astrocytes, lack of collagen and Schwann cells) and functionally (similar neurotransmitters), the ENS can be considered analogous to the central nervous system (CNS). It contains sensory, inter and motor neurons and has as many nerve cells as the spinal cord. This highly integrated system, which controls motility and modulates bowel secretion and vascular perfusion has been called the *"gut brain."* It seems to have an important role also in the modulation of local immune phenomena and inflammatory responses occurring in the gastrointestinal tract. The large amount of mucosal associated lymphoid tissue is continuously challenged by dietary antigens, toxins, and bacteria and interacts with the ENS to generate motor and immune responses, often leading to expulsion of the offending agent (emesis or diarrhea).

Unlike other autonomic ganglia that have mainly a relay-and-distribution function for signals sent from the brain and spinal cord, the ENS has the unique characteristic to be programmed to perform most of its tasks in the absence of CNS control. For example, the peristaltic reflex and the cycling of migrating motor complexes (MMCs) occur unchanged in a bowel deprived of extrinsic innervation. The ability to maintain most of its function independently of CNS control has fostered extensive laboratory research of chemical mediation of reflexes, mechanisms of depolarization, nerve cell migration, development, and differentiation, leading to knowledge that has already made the transition to application in clinical practice.

The increased interest in the ENS and its relationship with the CNS has brought together scientists from different disciplines generating the field of *Neurogastroenterology* (to emphasize such close relationship, the Journal of the American Motility Society has recently changed its name to "Neurogastroenterology and Motility." The increased understanding of the neurobiology of the gastrointestinal tract is also allowing a better understanding of some of the functions of the "other" brain, the CNS. For example, much has been learned from the study of *neuronal plasticity* in the ENS, the change in neuronal structure and function in response to alterations in input. Long lasting visceral hypersensitivity in irritable bowel syndrome has been noted to derive from repetitive high-pressure contractions or mucosal inflammatory responses to infections. Adaptation to vagotomy, bowel resection and transplantation offer other examples of plasticity of the ENS. There is also evidence that memory of early events may also affect the long-term responses of the ENS to stress predisposing to functional bowel disorders.

In the past few years much attention has been centered on the role that the interstitial cells of Cajal (ICC) and nitric oxide (NO) have in coordinating gastrointestinal motility and mediating sphincter function.

Interstitial cells of Cajal

The ICC have characteristics making them a hybrid between nerve and muscle cells. Like the smooth muscle cells, the ICC derive from mesenchymal precursors. They are interposed within the gut musculature and connect with nerves and smooth muscles. The ICC communicate with each other and the smooth muscle through long processes via gap junctions forming extensive networks of electrically coupled cells. They are also present at the level of the sphincters. Certain groups of ICC are currently regarded as the source of the spontaneous slow waves of the gut musculature *(pacemaker cells).* The slow waves of depolarization constitute the basic underlying mechanism allowing the smooth muscle cells to contract and control frequency and propagation of contractions. Hence, their designation as a pacemakers. Evidence for the role of ICC as pacemakers derives from the observation that: 1) ICC are located in regions where electrical slow waves are generated. 2) Isolated ICC have been shown to generate pacemaker activity. 3) Removing or uncoupling ICC from the smooth muscle leads to disruption of the slow wave activity and causes abnormal gut motility. Without ICC it is impossible to pace smooth muscle cells to produce slow waves. Other ICC appear to be involved in the transduction of the relaxation of smooth muscle triggered by nitric oxide.

The tyrosine-kinase receptor c-*kit* has been found to be a specific marker for ICC. C-*kit* is a receptor tyrosine kinase activated by the stem cell factor (SCF). Immunohistochemical studies using kit antibodies are expanding our knowledge about the structure of ICC networks, the interactions of ICC with other nerve or muscle cells, and the clinical consequences of losing or damaging the ICC. Mice with defective c-*kit*/SCF system do not develop ICC, have a disorganized motility pattern, and are not able to tolerate enteral feedings. Recent studies indicate that the distribution of ICC is abnormal in achalasia of the esophagus, hypertrophic pyloric stenosis and Hirschsprung's disease, suggesting that these conditions may share a common pathophysiologic defect. Other motility disorders, including pseudo-obstruction, colonic inertia, and gastroparesis may be related to abnormalities in ICC. There have been reports of delayed maturation of ICC contributing to neonatal pseudo-obstruction. However, caution should be used not to overinterpret the role of ICC until more is known about the age and site specific characteristics of these cells and in the absence of studies that show a causal rela-

tionship between abnormal function and abnormal ICC.

Nitric oxide

Nitric oxide is a lipid-soluble gas generated from the oxidation of the amino acid arginine to citrullin by NO synthase enzymes. It has a very short half-life, cannot be stored, is highly permeant, and moves from the site of production to sites of action by simple diffusion. Nitric oxide has now been recognized as the primary neurotransmitter of nonadrenergic-non-cholinergic nerves, which mediate the majority of inhibitory responses in the gastrointestinal tract. Vasoactive intestinal peptide (VIP) and NO interplay and amplify each other's action to control gut muscle relaxation. Nitric oxide seems to have an important role in coordinating esophageal peristalsis, LES relaxation, gastric accommodation, sphincter of Oddi opening, and internal anal sphincter relaxation. Inhibitory neurons to the circular muscle are continuously active, with muscle contractions occurring only when they are switched to inactive state. In sphincters, the inhibitory neurons are normally inactive and are activated only when the sphincter needs to open, usually in coordination with contractile events in the proximal regions.

The absence or the overexpression of NO leads to abnormal motility. Inhibitors of NO synthase accelerate intestinal and colonic motility, decrease number of LES relaxations, increase gastric and gallbladder emptying, and attenuate perception of visceral nociceptive stimuli. Deficiency of NO is found in achalasia, pyloric stenosis, and Hirschsprung's disease. It is conceivable that NO and NO inhibitors will be able to be used in the future for the treatment of gastrointestinal motility and functional bowel disorders. Currently, non-specific activation or inhibition of this ubiquitous molecule may cause undesired neurological, cardiovascular, and immunological side effects, making its use as a therapeutic agent problematic.

ENS and CNS interaction *("gut feelings")*

We have emphasized so far the important role of the ENS in generating and regulating gastrointestinal motility. Other motor activities, such as the substitution of the fasting with the postprandial motility pattern are under the influence of extrinsic–in this case, vagal–innervation. Patients who have had a vagotomy or have undergone small bowel transplantation suffer some of the consequences of such abnormal motility. After vagotomy the stomach loses some of its accommodative relaxation (it becomes "stiffer"), has a delayed emptying of solids and an accelerated emptying of liquids. Children who have received an isolated small bowel or multivisceral transplantation and have a gut completely devoid of extrinsic innervation, struggle for years with control of diarrhea partially because the MMCs persist after feeding, leading to a faster intestinal transit.

It is common experience that our brain may cause our gut to become hyperactive (for example, under periods of emotional stress or in response to painful stimuli). It is now known that the amount of information departing the gut and reaching the CNS greatly outnumbers the opposite traffic. The vagal, splanchnic, and pelvic nerves are estimated to have afferent-to-efferent fiber ratios of 9:1, 3:1, and 1:1 respectively. Just as the other large external surface, the skin, is regarded as a sensory organ, so should the intestinal mucosa. How the gut expresses its feelings (and the ENS and CNS talk to each other) has been a subject vastly investigated in the past few years. Afferent activity from the gastrointestinal tract rarely reaches the level of consciousness and is primarily involved with regulatory processes and coordination of digestive functions. However, visceral sensation does occur and can be subdivided into two broad categories: (a) nonpainful conscious sensations, which provide the individual with vague feelings, such as fullness, hunger, urgency, satiety, or nausea, and (b) painful visceral sensation, which informs the individual about potentially noxious events. The latter usually leads to activation of the autonomic nervous system aimed at expulsion of the noxious stimulus. Intestinal distention seems to be the stimulus for visceral pain in the majority of patients.

It is now well established that in functional bowel disorders (FBD) there is an altered afferent threshold for both noxious and physiologic stimuli (hyperalgesia and allodynia). Such evidence derives from studies using the barostat and the tensostat: instruments aimed at distending different areas of the gastrointestinal tract with a variety of closely monitored pressures and volumes. The mechanism by which such hypersensitivity occurs is unclear. There may be 1) a lower threshold to activate sensitized mechanoreceptors; 2) abnormal brain processing of accurate information arriving from the periphery, or 3) both. The use of PET scanning and/or functional MRI has recently demonstrated that patients with FBD activate different regions of the brain compared to controls in response to painful distensions of the esophagus or in anticipation of painful rectal stimulation. The *limbic system*seems to be a critical region for the integration of specific discrimination of pain, emotional experience, and decision-making processes. An increased understanding of the neurobiology of emotions and how gut and brain interact may clarify in the future the mechanisms of action of mood affecting medications on symptoms of patients with FBD and the close association between certain affective disorders and FBD.

How has all this wealth of new information impacted our care of patients with gastrointestinal motility disorders?

Enteric neuropathies

Pathophysiology

Damage to the ENS, either congenital or acquired, causes a wide variety of disorders, with different degrees of severity and phenotypic presentation. The study of discrete, stereotypical motor disorders, such as achalasia, hypertrophic pyloric stenosis, and Hirschsprung's disease has provided information that may be extrapolated to enhance our understanding of the more generalized types of motility disorders, such as pseudo-obstruction. Being that each of these conditions is characterized by a non-relaxing sphincter, it is not surprising that the inhibitory neurotransmission is altered. In all these conditions there is a loss of nitrergic and VIP containing neurons in the affected seg-

ments. It has been suggested that in Hirschsprung's disease the lack of intramuscular nitrergic nerves results in sustained contraction of the aganglionic bowel. Other investigators have reported that the activity of NADPH diaphorase, normally present in many nerve fibers of the pyloric musculature, is selectively absent from the hypertrophied circular musculature of infants with pyloric stenosis. NO synthase-gene-deleted knockout mice develop pyloric hypertrophy with gastric outlet obstruction. Animal studies have also shown that loss of NO containing inhibitory neurons results in decreased or absent LES relaxation and decreased amplitude of swallow induced esophageal contractions. Studies of patients with achalasia have demonstrated absence of NO synthase in the LES, with LES relaxation occurring when NO is added to muscle strips.

The important role of the ICC in generating and coordinating motility has already been discussed. In hypertrophic pyloric stenosis (HPS) there seem to be underdeveloped ICC. The motor abnormalities in HPS are probably due to a combination of lack of inhibitory innervation (lack of NO containing nerves) and defective myogenic control caused by the absence of ICC. Similar mechanisms have been postulated for the pathogenesis of Hirschsprung's disease and achalasia. Of interest is the observation that two cases of resolved HPS were associated with normalization of ICC distribution.

There are increasing reports of ICC abnormalities in pseudo-obstruction and it has been proposed that the etiology of this condition be divided in myopathic, neuropathic and *ICCpathic*. Often ICC abnormalities are seen in conjunction with neural abnormalities, making the role of ICC difficult to ascertain. Moreover, a normal number of ICC may be associated with an abnormal ultrastructure of ICC, as determined by electron microscopy. Establishing a national tissue bank is a very high priority if we wish to enhance our understanding of the pathophysiology of pseudo-obstruction and other generalized motility disorders, rare conditions likely to have heterogeneous etiologies.

Genetics

The study of the ontogeny of the ENS has led to an increasing understanding of the genetics of some forms of motility disorders. Several genetic markers for familial and sporadic forms of Hirschsprung's disease have been identified. It has become clear that Hirschsprung's disease is a multigenic disorder with incomplete penetrance and variable expressivity. Mutations in the chromosome 10 mapped RET proto-oncogene have been associated with long segment aganglionosis and are found in multiple endocrine neoplasia (MEN) 2A, MEN 2B, papillary thyroid carcinoma, and familial medullary thyroid carcinomas. Endothelin-B receptor genes and endothelin-3 genes have been recognized as susceptibility genes for the shorter forms of Hirschsprung's disease and are found in type 2 Waardenburg syndrome. Other gene abnormalities have been identified in smaller subgroups of patients. All the genetic defects identified so far account for about 50% of all forms of Hirschsprung's disease. These genetic abnormalities are thought to cause impaired neuronal crest migration and differentiation. They may constitute a model for the study of the development of more generalized forms of motility disorders. Identification of these and other genetic markers may permit in the future prenatal diagnosis of motor disorders. As a consequence of the incomplete penetrance and the notion that in many sporadic cases the responsible gene has not been identified, prenatal diagnosis of Hirschsprung's disease is not recommended yet.

Treatment

Up to the early 1970s, epinephrine and acetylcholine were considered the main neurotransmitters of the sympathetic and parasympathetic system in the gastrointestinal tract. Serotonin (5HT) was then identified as produced and effective in the ENS with approximately 95% of 5HT found in the ENS and 5% in the CNS. Since that time many other molecules have been found to work as neurotransmitters leading to the development of several promising drugs for the treatment of gut motility and sensory abnormalities. Identification of 5-HT receptors, particularly 5-HT3 and 5-HT4 receptors, has led to a number of studies of 5-HT3 (Ondansetron, Granisetron, and Alosetron) and 5-HT4 antagonists. Both classes of drug appear to block intestinal sensory receptors, decrease motor activity in the distal intestine, and increase colonic compliance. These agents are beginning to find a role in the treatment of women with diarrhoea predominant IBS. Partial or selective 5-HT4 agonists (Tegaserod, Prucalopride) may improve constipation or constipation-predominant IBS by normalizing bowel habit and reducing abdominal pain.

Alternative approaches to reducing visceral sensation include the use of the opioid kappa agonists (trimebutine, fedotozine), that have no central opioid effects. Preliminary clinical trials have so far provided conflicting results in the treatment of IBS and nonulcer dyspepsia. Other new approaches to modify intestinal motility include the development of gut selective muscarinic M3 receptor antagonists such as zamifenacin and darifenacin. Finally, octreotide reduces the perception of rectal distension and accelerates intestinal transit.

Increased understanding of neural mechanisms underlying transient lower esophageal sphincter relaxations, the main pathophysiologic mechanism causing gastroesophageal reflux, has led to hypothesize the use of GABA beta agonists and atropine in the treatment of this condition. More data are needed before such treatments may be recommended in children.

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Notes

Healing gastrointestinal mucosa: Two sides to the problem

Daniel Podolsky, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will be able to:

1. Define the major stages in healing of superficial and deep mucosal injury and their relationship to a variety of disorders affecting the gastrointestinal tract throughout its length;

2. Define the role of non-peptidyl and peptidyl factors in mucosal healing;

3. Define the variety and actions of different classes of regulatory peptides (growth factors, cytokines and Trefoil Peptides) in promoting mucosal repair;

4. Characterize the mechanisms of action of the pro-healing effects of regulatory peptides;

5. Describe potential therapeutic novel approaches to enhance healing of GI mucosal damage irrespective of the cause of the initial injury.

Notes

Obesity and the Regulation of body weight and appetite

Lee Kaplan, MD, PhD

LEARNING OBJECTIVES

At the conclusion of this session, participants will be able to:

1. Understand the changing patterns and clinical significance of obesity in children and adolescents;

2. Understand the contributions of genetic, environmental and psychological factors to human obesity;

3. Understand the basic mechanisms of weight and energy regulation and their implications for effective obesity therapy;

4. Understand the current therapeutic options for the treatment of obesity and its sequelae.

Obesity and the Regulation of Body Weight and Appetite

Lee M. Kaplan, MD, PhD

Introduction

In the past few years, several factors have conspired to generate renewed interest in obesity within the medical and scientific communities:

Epidemiology: Numerous studies published during the past decade have demonstrated that excess body fat is associated with substantially increased morbidity and premature mortality. Although much of this morbidity results from diabetes and atherosclerotic cardiovascular disease, several GI and hepatic disorders contribute as well.

Epidemiological analysis has further shown that the prevalence of obesity in adults and children in the U.S. has increased 50% during the past twenty-five years. These observations have led to a renewed emphasis on the prevention and treatment of obesity by public health agencies and authorities.

Genetics: Several lines of epidemiological evidence suggest that genetic factors account for up to 80% of an individual's ***predisposition*** to developing obesity. The discovery during the past five years of specific genetic defects that **lead to** or provide **protection against** obesity in animals and humans has increased our understanding of the important role of genes in this disorder.

Recent genetic discoveries have also illuminated the biological mechanisms and metabolic pathways that underlie obesity and have provided several new targets for intervention and drug discovery. These developments have led to accelerated activity within the biotechnology and pharmaceutical industries and have generated great enthusiasm within the scientific community. At present, there are more than 120 new drugs undergoing evaluation for obesity treatment, with more than 40 in early clinical development.

Therapies: The introduction of dexfenfluramine (Redux®) and the combination of fenfluramine and phentermine ("fen/phen") in the mid-1990s led to renewed excitement and hope among physicians and their patients with obesity. The availability of these apparently effective pharmaceutical agents and the associated marketing efforts substantially increased public understanding of obesity as a serious medical disorder.

Although the withdrawal of these agents was met with disappointment and considerable disillusionment, enhanced appreciation of the medical consequences of obesity has remained, providing further support for ongoing drug development efforts. The current absence of effective, long-term pharmacological solutions for this medical problem has led to renewed examination and appreciation of the utility of surgical approaches to obesity management. As a result, Roux-en-Y gastric bypass is now the most common upper GI surgical procedure performed in the U.S. and the fastest growing of all general surgical procedures.

Definitions

Obesity is defined as excess body fat. In most clinical settings, body mass index (BMI) is a fairly reliable predictor of body fat. BMI is a ratio between an individual's weight and the square of his or her height and is reported as kg/m2. The following formula can be used to convert from English to metric units:

$$\text{BMI (in kg/m2)} = \frac{\text{Weight (lbs.)}}{[\text{height (in.)}]^2} \times 704$$

The normal BMI for adults (that BMI associated with the lowest morbidity and mortality) ranges from approximately 18 to 25. According to 1998 standards established by the U.S. Public Health Service and the World Health Organization, *overweight* is defined as a BMI between 25 and 30 and *obesity* is defined as a BMI > 30. Obesity is further subdivided by severity into class I (BMI = 30-35), class II (BMI = 35-40) and class III (BMI > 40).

The normal BMI for children varies with age. Standard BMI curves for children and adolescents have recently been published. These are based on the BMI distribution for children of different ages during the 1960s. Using that standard, individuals with a BMI between the 85th and 95th percentile are considered as being *at risk* for becoming overweight, and those with a BMI above the 95th percentile are considered *overweight.*

Epidemiology

The average BMI of U.S. adults is now approximately 27 kg/m^2, and more than half of American adults are overweight. Nearly one-fourth of us (23%) have obesity, up from only 16% as recently as 1980. According to data from the CDC, the prevalence of obesity among U.S. adults increased each year during the 1990s. Obesity is particularly prevalent in selected population subgroups: more than 50% of African-American and Mexican-American women have this disorder.

Obesity in children and adolescents, which strongly correlates with the later development of adult obesity, is also on the rise. Recent studies indicate that 13% of U.S. children are overweight or obese, up from 7% as recently as 1980 and 5% in 1964. Positive predictors of obesity in children include the BMI of each parent, the hours of TV watching daily, total food intake, and percentage of calorie intake in the form of protein or total fat. Negative predictors include family income and family size.

These rapid changes demonstrate that despite the importance of genetics in determining predisposition to obesity, development of obesity itself is strongly influenced by environmental cues. Such cues likely include (1) the overall decrease in physical activity, (2) the increased availability, caloric density, processing, fat and/or carbohydrate content and homogeneity of consumed foods, (3) the increasingly chaotic patterns of eating, and (4) the increased stress and pace of life (including eating). The increasing prevalence of obesity in children supports the prediction that this epidemic has not yet reached its peak.

Obesity is the second leading cause of premature death in the U.S. after tobacco use. Approximately 300,000 Americans die annually as a result of obesity-related disease (vs. 450,000 for tobacco). More than 30 distinct diseases are promoted, enhanced or accelerated by obesity. A tetrad of these disorders known as metabolic syndrome or "syndrome X," including type 2 diabetes mellitus, hypertension, hypercholesterolemia and atherosclerotic cardiovascular disease, is responsible for a large portion of obesity-associated morbidity and mortality. The prevalence of each of these disorders (as well as those listed below), as well as overall mortality, show a strong positive correlation with BMI in both men and women.

Obesity accounts for approximately $100 billion of health care expenditures annually in the U.S. (approximately 7% of all health care costs). Consumers spend an additional $45 billion on weight loss goods and services, and an estimated $10 billion is lost yearly because of obesity-associated decreases in workplace productivity.

The primary gastrointestinal and hepatic diseases caused or facilitated by obesity include nonalcoholic steatohepatitis (NASH), gallstones, gastroesophageal reflux disease (GERD), colon cancer and esophageal adenocarcinoma. It is estimated that 75% of the recent increase in the incidence of adenocarcinoma of the esophagus results from the increased prevalence of obesity, the associated GERD and the resulting dysplasia.

Other obesity-associated diseases include sleep apnea (both obstructive –type and central hypoventilation syndrome), hypertriglyceridemia, venous stasis, venous thrombosis, cellulitis, asthma (secondary to GERD), axial arthritis, gout, infertility, depression and anxiety disorders, and four additional types of cancer (breast, endometrial, ovarian and prostate).

Obesity also exacts a severe socioeconomic toll. Whether because of physical and psychological effects, external prejudice or a combination of all three, obesity is associated lower educational achievement, increased unemployment and underemployment.

Biology

Body weight is tightly regulated, with caloric intake and energy expenditure normally matched to within a tolerance of 0.01%. Obesity results from the failure of this sophisticated regulatory system, with an imbalance between total energy intake and expenditure. It is induced by some combination of genetic, environmental and psychological influences.

Regulation of body weight appears to be coordinated primarily by the hypothalamus, which receives afferent information from several sources. Circulating levels of the adipocyte-derived hormone leptin signal body energy stores. Through neurohumoral mechanisms, the gastrointestinal tract provides information about recent food ingestion, and the cerebral cortex contributes behavioral information and regulation. Most people appear to maintain their weight around a physiological setpoint, and it is thought that obesity results in part from a disturbance in this control system (the mechanism of which is not known). Significant deviation from the setpoint leads to appropriate compensatory responses: thus weight loss generates a simultaneous drive for energy conservation (decreased expenditure) and increased caloric intake (increased hunger). Forced overeating generates the opposite effects.

The effector arm of hypothalamic control of body weight includes the regulation of food intake, metabolic activity and energy expenditure (primarily in muscle and fat tissue). Among the many mediators of these regulatory systems are neuropeptide Y, insulin, and the sympathetic nervous system.

It is likely that obesity represents the manifestations a spectrum of different disorders, each arising from distinct defects (or groups of defects) in the cortical (including psychological), hypothalamic, endocrine and/or metabolic components of the weight regulatory system.

Genetics

Evidence for a strong genetic contribution to human obesity comes from a variety of sources, including twin and familial aggregation studies. These studies suggest that genetic factors account for 60 to 80% of the predisposition to obesity. One such study calculated the correlation between BMIs of related pairs. For genetically unrelated spouses, the BMI correlation was 10%. For first cousins it was 14%. For parent-child pairs and siblings, the correlation was approximately 25%, and for monozygotic twins, it was 88%.

Other support for the genetic basis of obesity comes from animal studies. There are several naturally-occurring models of weight dysregulation in mice and rats, including single-gene defects that lead inexorably to obesity and polygenic traits that affect the susceptibility of these animals to diet-induced obesity. The defect in one of these strains, the obese (ob/ob) mouse, was shown by Friedman and colleagues to reside in the gene encoding leptin, a circulating hormone secreted by fat cells that signals the level of energy reserves (body fat) to the hypothalamus. The obese mice fail to synthesize leptin. The hypothalamus interprets the absence of leptin as severely diminished energy stores, generating a coordinated response that includes increased food ingestion and decreased energy expenditure. Replacement of the missing leptin reverses these effects. Identification of the defects in other strains of genetically obese mice and rats has revealed several additional components of the weight regulatory system. Similar studies have also identified the mechanisms underlying resistance to obesity exhibited by other naturally-occurring and engineered strains of mice.

Although a few individuals with severe obesity have been shown to lack leptin, nearly all obese individuals exhibit an excess of circulating leptin in direct proportion to their BMI. **Thus, human obesity appears to result from functional resistance to the effects of leptin,** much as type 2 diabetes reflects resistance to the physiological effects of insulin. Not surprisingly, therefore, early clinical trials have shown little effect of leptin to decrease body weight in obese individuals. The mechanisms of leptin resistance in human obesity are currently unknown.

Treatment

The goals of obesity management are (1) to ameliorate or reverse the complications of obesity, (2) to improve cardiovascular fitness and muscle strength, and (3) to improve physical and psychological well-being. Several approaches can be used to achieve these goals, including (but not limited to) weight loss.

The primary approaches to the treatment of obesity include (1) dietary modification, (2) exercise, (3) behavioral therapy, (4) pharmacotherapy, and (5) weight loss surgery.

Dietary modification

Dietary modification promotes weight loss by decreasing total caloric intake and generating a mild calorie deficit. There is no objective evidence that variation in the proportion of calories obtained from fats, carbohydrates and proteins has significant impact on the efficacy of weight loss diets. In fact, many investigators believe that the primary effect of dietary intervention is to impose structure to often chaotic eating patterns. Most diets generate short-term success; long-term success is rare with any of them. Although high-fat diets have been recently popular, they suffer from the associated increased risk of hyperlipidemia and its consequences.

The goals of dietary therapy are to produce an energy deficit of 500-1000 kcal/day, leading to a weight loss of no more than 1-2 pounds/week. Very low calorie diets (<800 kcal/day total) are not recommended. They are no more effective than less invasive approaches and have been associated with a significantly increased risk of adverse effects.

Exercise

Exercise confers multiple benefits in obesity management. Both aerobic and anaerobic exercise promote increased lean body mass, which leads to increased resting energy expenditure. Several recent studies have demonstrated that frequent aerobic exercise is associated with decreased overall mortality, independent of initial BMI or weight loss. Several mechanisms likely contribute to this effect, including improved cardiovascular function, improved blood lipid profile, decreased blood pressure and glucose, and an improved sense of well-being.

Behavioral therapy

Behavioral therapy is aimed at promoting sustained changes in patient eating and exercise patterns. The major approaches to behavioral therapy are (1) self-monitoring of dietary choices, eating and exercise behaviors,(2) stimulus control to minimize the risk of maladaptive behaviors, and (3) reinforcement of successful activities. Long-term success of behavioral therapies is dependent on the frequency and intensity of behavioral counseling and support, rather than the specific therapeutic approach or professional training of the counselor. Because of the high cost of intensive, long-term behavioral therapy, we have recently developed a web-based program to extend on-site counseling. We are currently testing the efficacy of this program in a controlled clinical trial.

Pharmacotherapy

Several medications have been approved by the FDA for treatment of "clinically-significant" obesity, including phentermine (Ionamin®, Fastin® and others), sibutramine (Meridia®), and orlistat (Xenical®). [Clinically significant obesity is defined as BMI > 30 or BMI = 27-30 with one or more obesity-related disorders]. Unfortunately, the clinical efficacy of these drugs is limited. For each, total weight loss averages less than 5% of initial body weight. A small minority of patients lose considerably more weight, although we cannot currently predict who those individuals will be. Moreover, all of these agents are approved only for short-term use (3-12 months total), further limiting their clinical utility. Because initial weight loss induced by diet, exercise and behavioral therapies is generally as effective with and without medications, routine use of these agents is not indicated.

Since they can only be used for limited periods of time, the strongest indications for these agents appear to be in situations requiring short-term weight loss, even if transient. Examples include (1) weight loss to reduce the risk of an upcoming elective surgical or endovascular procedure and (2) acute weight loss to reverse one or more immediately life-threatening complications of obesity (especially obstructive sleep apnea or heart failure). These agents may also be useful as an adjunct to other therapies for patients who experience transient difficulties maintaining weight loss (although their short-term indication limits utility in this situation as well). For phentermine and sibutramine, which affect CNS regulation of appetite and eating behavior, there may be additional utility in reinforcing behavioral modification after patients have completed acute weight loss and entered the weight maintenance phase. The efficacy of short-term treatment during this period is currently under investigation.

Phentermine

The safe half of the "phen/fen" combination, phentermine (Ionamin®; Fastin®) is a mild adrenergic agent without addictive properties. It acts by stimulating norepinephrine release at nerve terminals. It is a weak appetite suppressant (anorexigen) and is approved only for 3-month use. Potential adverse effects include hypertension and palpitations.

Propanolamine

Propanolamine (Dexatrim® and others) is a weak adrenergic agent sold over-the-counter as an anorexigen. It has no proven efficacy for short- or long-term weight loss beyond that provided by nonpharmacological approaches.

Sibutramine

Sibutramine (Meridia®) is a combined serotonin and norepinephrine reuptake inhibitor that exhibits modest anorexigenic

properties. Its combined effect on serotonergic and adrenergic pathways is reminiscent of the (more powerful) effects of the phen/fen combination. Unlike fenfluramine and dexfenfluramine (Redux®), however, sibutramine has not been associated with heart valvular problems or pulmonary hypertension. Its primary adverse effect is blood pressure elevation, and it is contraindicated in patients with uncontrolled hypertension. Sibutramine is currently approved for use up to 12 months. Safety and efficacy trials of long-term (5-year) sibutramine therapy are currently underway; however, modest regain of weight while on this drug is already observed between 12 and 24 months of therapy.

Orlistat

Orlistat (Xenical®) is a selective pancreatic lipase inhibitor that inhibits intestinal fat digestion. Depending on dietary composition, it can inhibit the absorption of up to 30% of ingested fat. Its short-term efficacy in promoting weight loss is similar to that of sibutramine and phentermine (approximately 4-5% weight loss at one year). However, like these unrelated agents, significant weight regain is observed between 12 and 24 months of continued use. The major adverse effects of orlistat are steatorrhea with oily leakage and malabsorption of fat-soluble vitamins. Patients treated with this agent should receive supplements of vitamins A, D, E and K and monitored closely for evidence of deficiencies in these vitamins, vitamin B12 and iron.

Other psychotropic agents

Several classes of psychotropic agents can affect body weight. Many, including olanzapine (Zyprexa®), dipropylacetic acid (Depakote®), and paroxetine (Paxil®) are frequently associated with significant weight gain. Significant weight loss has been observed during treatment with others, including topiramate (Topamax®) and bupropion (Wellbutrin®). Topiramate has recently been used in selected patients to counteract the prominent weight gain associated with other psychotropic agents. Clinical trials to determine the therapeutic efficacy of topiramate and bupropion are currently underway.

The effect of the selective serotonin reuptake inhibitors (SSRIs) is variable: most patients experience little weight change, some gain weight (most commonly seen with paroxetine), and a few lose weight. For those few, these agents may be useful. As with the other agents described above, however, the anorexigenic effects in most patients are weak and relatively short-lived.

Weight loss surgery

During the past 25 years, gastric surgery has been shown to be the most effective approach for generating long-term weight loss. More than 90% of patients experience significant (>20-25%) weight loss, and between 50 and 80% maintain weight loss over 5 years. (In contrast, the 5-year efficacy of other approaches is between 5 and 10%).

Two surgical procedures are most commonly used to treat obesity: Roux-en-Y gastric bypass (RYGB) and vertical banded gastroplasty (VBG). VBG is a restrictive procedure in which a band is placed below a small proximal gastric pouch that limits the acute storage capacity of the stomach.

RYGB creates a similarly small gastric pouch as the afferent component of a gastrojejunostomy. Food enters the jejunum directly from the stomach. The distal stomach is completely excluded from the food flow, emptying (with the pancreatico-biliary secretions) into the distal bowel through the Roux-Y limb.

Despite their long use, the mechanism by which these procedures effect weight loss are not completely known. Both procedures effectively limit the storage capacity of the stomach, and RYGB can occasionally lead to transient dumping syndrome, suggesting that some of their therapeutic effects result from negative reinforcement leading to behavior modification. Because RYGB can be associated with vitamin and mineral deficiencies, malabsorption has been thought to contribute to the observed weight loss. This effect is minor in most patients, however.

Patients who undergo these procedures experience a sharp decrease in appetite, however, which appears to be a dominant contributor to the resulting weight loss. The mechanisms of this effect are unclear, but likely include stimulation of central satiety pathways. In both procedures, this effect may occur from rapid distention of the gastric pouch with small meals. RYGB likely generates additional satiety signals resulting from the rapid transfer of undigested nutrients into the jejunum.

Accepted indications for surgical weight loss therapy are (1) BMI > 40 or BMI = 35-40 with one or more obesity-related disorders, (2) previous unsuccessful nonsurgical attempts at long-term weight loss. Weight loss surgery is most effective when accompanied by pre- and post-operative comprehensive therapy to modify eating and exercise behavior. Such adjunctive therapy increases the likelihood of long-term success by as much as 50% and should be a standard component of surgical obesity management.

Overall mortality from weight loss surgery is approximately 0.5%. Major early complications include wound infection, venous thrombosis, gastric ulcer, dehydration, malnutrition and atelectasis. In patients with hypoventilation syndrome or obstructive sleep apnea, care must be taken to avoid CO2 retention. The most common late complications of weight loss surgery are B12 and iron deficiencies, occurring in as many as 30% of patients unless supplements are given. Incisional hernias frequently develop in patients after wound infections. Other potential complications include cholecystitis secondary to gallstone formation and staple line breakdown. Dumping syndrome is rare if patients avoid high concentrations of simple carbohydrates. When present, it usually resolves within several months; if severe, it can usually be controlled with low doses of octreotide.

Multiple minor variations of VBG and RYGB are used in different centers. Other surgical procedures have been used to promote weight loss in the past, but have been discarded. Most notably, the jejunoileal bypass, a weight loss procedure used in the 1960s and early 1970s, is associated with a high incidence of microvesicular steatosis and liver failure. Current procedures have not been associated with this complication.

Future therapies

Obesity is currently an active field for pharmaceutical development, with more than 120 agents at various stages of development. Although most of these compounds are still in preclinical development, several have progressed to clinical trials. The mechanisms of action of these drugs are varied, and they act at several different sites in the weight regulatory machinery. As a result, it is likely that one or more of them will be far more effective, at least for a subset of patients with obesity, than currently available agents. We and others are participating in clinical trials of several of these newer drugs.

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Immunobiology of the gastrointestinal tract: Food induced hypersensitivities

Scott H. Sicherer, MD

LEARNING OBJECTIVES:

At the conclusion of this session, participants:

1. Should be familiar with the signs and symptoms of the various food-induced gastrointestinal hypersensitivities;

2. Should be familiar with the underlying immunopathogenic mechanisms believed responsible for these disorders;

3. Should gain a better understanding for the complexities of diagnosing and managing patients with gastrointestinal hypersensitivities.

Immunobiology of the Gastrointestinal Tract: Food-induced Hypersensitivities

Scott H. Sicherer, MD

The prevalence of food allergy is greatest in the first few years of life, affecting up to 8% of infants under the age of 3 years[1] and then falling to about 1.5% of the population after the first decade. Gastrointestinal symptoms were reportedly most common in children undergoing food challenges, with diarrhea developing in 63% of children, vomiting in 38%, and colic in 19% of babies. In four prospective studies from four different countries, appropriately performed milk challenges revealed that 2.2% - 2.5% of infants had cow milk allergy in the first 1-2 years of life.[2] However, follow-up studies revealed that nearly 85% of these milk-sensitive infants lost their reactivity to milk by their third birthday.[3] Comparable controlled studies have not been performed in adults, so that the prevalence of food-induced gastrointestinal hypersensitivity in this population is not known.

The primary role of the gastrointestinal tract is to process ingested food into a form that can be absorbed and utilized for energy and cell growth. During this process, both non-immunologic and immunologic mechanisms operate as a "barrier" to block potentially harmful foreign antigens (bacteria, viruses, parasites, and food proteins) from entering the body proper. Immaturity of these mechanisms in infants (e.g. reduced basal acid output, altered antigen binding and transport, deficient secretory IgA) reduces the efficiency of the infant mucosal barrier resulting in increased antigen uptake.[4] However, even in the mature adult gastrointestinal barrier, early studies clearly demonstrated that immunologically intact food proteins enter the circulation and are distributed throughout the body.[5,6] Since intact food antigens penetrate the gastrointestinal tract and enter the circulation in both normal children and adults[3], it is imperative that the gut-associated lymphoid tissues [GALT] promote local and systemic 'tolerance' to these nutrient proteins.

Early studies of food hypersensitivity focused on roentgenologic changes associated with the ingestion of food allergens. In one of the first reports, fluoroscopy was utilized to compare the outcome of barium sulfate meals with and without food allergen in 12 food allergic children.[7] The most prominent findings included gastric hypotonia and retention of the allergen test meal, prominent pylorospasm, and increased or decreased peristaltic activity of the intestines. In the late 1930s, the rigid gastroscope was utilized to observe allergic reactions in the stomach.[8] Thirty minutes after a food allergen was placed on their mucosa, patients with gastrointestinal food allergy had markedly hyperemic and edematous patches of thick gray mucous and scattered petechiae at these sites.

Clinically, it is useful to categorize patients into those likely to have IgE-mediated food allergic disorders and those who have non-IgE-mediated disorders. IgE-mediated hypersensitivity reactions typically come on more quickly and foods responsible can often be determined by the presence of food-specific IgE antibodies (positive prick skin tests or RASTs).[9]

IgE-mediated food-induced gastrointestinal hypersensitivities

Several syndromes comprise the IgE-mediated, food-induced gastrointestinal hypersensitivities: oral allergy syndrome, gastrointestinal anaphylaxis, and subgroups of allergic eosinophilic gastroenteritis and infantile colic. The **oral allergy syndrome** is a form of contact allergy confined predominantly to the oropharynx and rarely involving other target organs. Symptoms include the rapid onset of pruritus and angioedema of the lips, tongue, palate, and throat, generally followed by a rapid resolution of symptoms, most commonly associated with the ingestion of various fresh fruits and vegetables.[10] Patients with birch or ragweed pollen-induced rhinitis are most frequently afflicted with this syndrome. In ragweed allergic patients, oral symptoms may be experienced following contact with various melons (watermelon, cantaloupe, honeydew, etc.) and bananas, whereas birch pollen allergic patients may develop symptoms following the ingestion of raw potatoes, carrots, celery, apples, hazelnuts and kiwi. Diagnosis is based upon a suggestive history and positive prick skin tests ("prick and prick," i.e. pricking the fresh fruit with a lance or needle and then pricking the skin of the patient with the implicated fresh fruits or vegetables).

Gastrointestinal anaphylaxis is a form of IgE-mediated hypersensitivity, which often accompanies allergic manifestations in other target organs and results in a variety of symptoms.[11] Symptoms typically develop within minutes to two hours of consuming a food and consist of nausea, abdominal pain, cramps, vomiting, and/or diarrhea. In some infants, the frequent ingestion of a food allergen appears to induce partial desensitization of gastrointestinal mast cells resulting in a subclinical reaction, with the only complaints being poor appetite and periodic abdominal pain. Diagnosis is established by clinical history, prick skin testing, resolution of symptoms following complete elimination of the suspected food, and recurrence of symptoms following oral food challenges.

Allergic eosinophilic gastroenteritis is characterized by infiltration of the esophageal, gastric and/or intestinal walls with eosinophils, an absence of vasculitis, and peripheral eosinophilia in about 50% of patients. The eosinophilic infiltrate may involve the mucosal, muscular, and/or serosal layers of the stomach or small intestine.[12] Eosinophilic invasion of the muscular layer leads to thickening and rigidity of the stomach and small intestine whereas infiltration of the serosal area commonly results in ascites containing eosinophils. Patients with

this syndrome commonly present with post-prandial nausea and vomiting, gastroesophageal reflux, abdominal pain, diarrhea, occasionally steatorrhea, and weight loss in adults or failure to thrive in young infants.[13] In a subset of patients with allergic eosinophilic gastroenteritis, food-induced IgE-mediated reactions have been implicated in the pathogenesis of this disorder. Patients with food-induced symptoms generally have atopic disease, elevated serum IgE concentrations, positive prick skin tests to a variety of foods and inhalants, peripheral blood eosinophilia, iron deficiency anemia, and hypoalbuminemia. Diagnosis is dependent upon the gastrointestinal biopsy demonstrating a characteristic eosinophilic infiltration. Elimination of the responsible food allergen from the diet for up to 12 weeks may be necessary to bring about resolution of symptoms and normalization of intestinal histology.

Non-IgE-mediated food-induced gastrointestinal hypersensitivities

The majority of food hypersensitivity disorders are not IgE-mediated, but are believed to be the result of various cell-mediated mechanisms. Consequently, tests for evidence of food-specific IgE antibodies are of no value in identifying the responsible food. Unfortunately, no laboratory tests have proven useful for identifying the foods provoking these disorders. These non-IgE-mediated hypersensitivities may be divided into the following syndromes: food-induced enterocolitis, food-induced eosinophilic proctocolitis, food-induced enteropathy, and the majority of allergic eosinophilic gastroenteritis. Table 1 summarizes the clinical features of these disorders.

Food-induced enterocolitis syndrome is a disorder most commonly seen in young infants presenting between 1 week and 3 months of age with protracted vomiting and diarrhea, which not infrequently results in dehydration.[14] Cow's milk and or soy protein are most often responsible, but enterocolitis secondary to egg, wheat, rice, oat, peanut, nuts, chicken, turkey and fish sensitivity have also been reported in older individuals.[15] Similar less severe reactions often are reported in adults to seafood (e.g. shrimp, crab, lobster). Stools frequently contain occult blood, polymorphonuclear neutrophils and eosinophils. Prick skin tests to the responsible food allergen are characteristically negative. Jejunal biopsies classically reveal flattened villi, edema, and increased numbers of lymphocytes, eosinophils, and mast cells. Foods challenges generally result in vomiting and diarrhea within 1 to 3 hours, and result in hypotension in about 15% of cases. The immunopathogenesis of this syndrome remains unknown, but some studies suggest that food antigen-induced secretion of tumor necrosis factor (TNF) from local mononuclear cells may account for the reaction.[16] Diagnosis can be established when elimination of the responsible allergen leads to resolution of symptoms within 72 hours and oral challenge provokes symptoms. Oral food challenges consist of administering up to 0.1 - 0.6 gm/kg body weight of the suspected protein allergen while monitoring the peripheral blood white count. Vomiting and diarrhea generally develop within 1 to 6 hours of challenge. In conjunction with a positive challenge, the absolute PMN count will increase at least 3500 cells/mm^3 at 4 to 6 hrs, and PMN's and eosinophils may be found in the stools. Food antigen challenges may lead to profuse vomiting, dehydration and hypotension, so they must be performed in a medical setting.

Food-induced eosinophilic proctocolitis (colitis) also generally presents in the first few months of life and is secondary to cow's milk or soy protein hypersensitivity, but about one-half of reported cases are now in breast-fed infants.[17] These infants usually do not appear sick, often have normally formed stools, and generally are discovered because of the presence of blood (gross or occult) in their stools. Blood loss is typically minor but occasionally can produce anemia. Lesions generally are confined to the distal large bowel and consist of mucosal edema with infiltration of eosinophils in the epithelium and lamina propria. Sigmoidoscopy findings are variable but range from areas of patchy mucosal injection to severe friability with small aphthoid ulcerations and bleeding. Diagnosis can be established when elimination of the responsible allergen leads to resolution of hematochezia, generally with dramatic improvement within 72 hours of appropriate food allergen elimination, but with complete clearing and resolution of mucosal lesions taking up to one month. Re-introduction of the allergen leads to resumption of symptoms within several hours to days.

Food-induced malabsorption syndromes (excluding celiac disease) often present in the first several months of life with diarrhea (not infrequently steatorrhea) and poor weight gain.[18] Symptoms include protracted diarrhea, vomiting in up to two-thirds of patients, failure to thrive, and malabsorption, demonstrated by the presence of reducing substances in the stools, increased fecal fat and abnormal d-xylose absorption. Cow's milk sensitivity is the most frequent cause of this syndrome, but it also has been associated with sensitivity to soy, egg, wheat, rice, chicken and fish. Patchy villous atrophy with cellular infiltrate on biopsy is characteristic of this disorder. Diagnosis is established by excluding the responsible allergen from the diet which should result in resolution of symptoms, although this may require several days to weeks. On endoscopy, a patchy villous atrophy is evident and biopsy reveals a prominent mononuclear round cell infiltrate and a small number of eosinophils, not unlike celiac disease, but generally much less severe.[19] Colitic features are usually absent, but anemia occurs in about 40% of affected infants and protein loss in the majority. Complete resolution of the intestinal lesions may require 6 to 18 months of allergen avoidance. Unlike celiac disease, loss of reactivity is reported to occur, but the natural history of this disorder has not been well studied.

Celiac disease is a more extensive enteropathy leading to malabsorption. Total villous atrophy and extensive cellular infiltrate are associated with sensitivity to gliadin, the alcohol-soluble portion of gluten found in wheat, oat, rye, and barley. Initial symptoms often include diarrhea or frank steatorrhea, abdominal distention and flatulence, weight loss, and occasionally nausea and vomiting. Oral ulcers and other extra-intestinal symptoms secondary to malabsorption are not uncommon. Villous atrophy of the small bowel is a characteristic feature of celiac patients ingesting gluten. Celiac disease is associated with the HLA-DQ2 (and DQ8) haplotype and

about 90% of celiac patients ingesting gliadin possess IgA anti-gliadin and anti-endomysium antibodies. Once the diagnosis of celiac disease is established, life-long elimination of gluten containing foods is necessary to control symptoms and to avoid the increased risk of malignancy.

Diagnosis is based upon a suggestive history and the demonstration of an eosinophilic infiltration of the gastrointestinal wall. Since the eosinophilic infiltrate may be sporadic, multiple sites should be biopsied. Elimination of suspect foods for 6-12 weeks should lead to resolution and normalization of gut histology, although clinical symptoms should improve substantially in 3 to 6 weeks. Challenge consists of reintroducing the suspect food allergen and demonstrating recurrence of symptoms and eosinophilic infiltrate on biopsy. If food allergens are not identified as provoking agents, oral steroids (at 1 to 2 mg/kg per day) are generally required to alleviate symptoms. Although symptoms usually respond to steroid therapy, exacerbations are not infrequent when steroids are discontinued. If exacerbations recur, a regimen of low dose prednisone or every other day prednisone may be successful in suppressing symptoms.[20]

Gastroesophageal reflux (GER) in young infants may be the result of food hypersensitivity. In a study of 204 infants < 1 year of age with gastroesophageal reflux (GER – diagnosed with 24 pH probe and esophageal biopsy), 85 (42%) were diagnosed with cow's milk-induced reflux by blinded milk challenges (as determined by 24 pH probe and esophageal biopsy). These infants experienced resolution of GER once cow's milk was eliminated from the diet.[21]

A number of gastrointestinal hypersensitivity disorders have been characterized, especially in young children. The true prevalence of these disorders remains to be determined. Although non-IgE-mediated mechanisms seem responsible for the majority of these disorders, the exact immunopathogenic mechanism(s) involved have not been elucidated. Table 2 summarizes a number of disorders that must be differentiated from food hypersensitivities.

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Table 1

Differentiating Non-IgE-mediated Food Hypersensitivities

	Food-induced Enterocolitis	Food-induced Eosinophilic Proctocolitis	Food-induced Malabsorption Syndrome	AEG
Age of onset	2 wk - 9 mo	1 wk - 3 mo	1 mo - 18 mo	1 mo & up
Duration	9 - 36 mo	6 - 18 mo	18 - 36 mo	1 yr & up
Food Proteins implicated	cow's milk soy	cow's milk soy breast milk[1]	cow's milk soy wheat barley	cow's milk egg, soy wheat barley
Clinical Features				
FTT or wt. loss	moderate	no	moderate	mod.- severe
Vomiting	prominent	no	variable	prominent[2]
Diarrhea	severe	rare	moderate	minimal
Hematochezia	moderate	mod. - severe	moderate	min. - mod.

AEG - allergic eosinophilic gastroenteritis

1 - food proteins in breast milk [most often cow's milk or egg protein]

2 - retching or "gastroesophageal reflux"

Table 2

Disorders Which Must be Differentiated From Food Hypersensitivities

Food Intolerances

Post-infectious malabsorption [secondary disaccharidase deficiency, villous atrophy, bile salt deconjugation]

Viral	Rotavirus
Parasitic	Giardia, Cryptosporidium
Bacterial	Shigella, Clostridium difficile

Metabolic Disorders

Transient fructose and/or sorbital malabsorption

Primary carbohydrate malabsorption — lactase deficiency, sucrase deficiency

Hypo- or Abetalipoproteinemia

Acrodermatitis enteropathica

Bacterial enterotoxins

Vibrio cholera, toxigenic E. coli, Clostridium difficile

Other Disorders

Cystic fibrosis

Chronic inflammatory bowel disease

"Chronic non-specific diarrhea of infancy"

Tumors - neuroblastoma [catecholamines or vasoactive inhibitory peptide]
Zollinger-Ellison Syndrome [gastrin]

Anatomical Abnormalities

Intestinal lymphangiectasia

Short bowel syndrome

Hirschsprung's disease [especially with enterocolitis]

Ileal stenosis

Notes

Hepatitis prevention and treatment

Mei-Hwei Chang, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will be able to:

1. Demonstrate the effect of universal hepatitis B vaccination program to enhance the understanding that "prevention is the most cost-effective way to control hepatitis virus infection";

2. Learn the indication, limitation, effect, and adverse reactions of antiviral therapy in those infected chronically by hepatitis B or C viruses.

Hepatitis Prevention and Treatment

Mei-Hwei Chang, MD

Key Concepts

1. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are both global health problems. They can cause acute, fulminant or chronic hepatitis, liver cirrhosis and even hepatocellular carcinoma. Chronic infection during infancy or early childhood has a longer disease course and thus requires special attention.

2. Universal HBV immunization is the most effective way to control HBV infection. It has reduced the prevalence of the chronic HBV infection rate to less than one tenth of the rate in the prevaccination era. The incidence of hepatocellular carcinoma (HCC) in Taiwanese children born after the program also was reduced.

3. Current main problems for the control of HBV infection and its related liver diseases are: (1) vaccine failure or unresposiveness, (2) misinformation of the side effect of HBV vaccine or ignorance, (3) relatively high cost of the vaccine for limited resources, (4) competition from other new vaccines.

4. The vaccine for HCV has not been successfully developed. Screening of blood products for anti-HCV has successfully reduced the post-transfusion HCV infection. Avoidance of parenteral exposure is also very important to prevent HCV infection.

5. Interferon injection and oral lamivudine therapy are the two currently available antiviral agents effectively inhibiting HBV replication. A meta-analysis revealed that 23% of the interferon-treated and 11% of the untreated children showed a complete response with hepatitis B e antigen (HBeAg) and HBV DNA clearance and normalization of aminotransferase levels. Lamivudine therapy in adults reavealed a 17% of HBeAg seroconversion rate after one year and a 27% response rate after two-year therapy. The long-term effect of lamivudine needs to be carefully observed in children.

6. For chronic hepatitis C, interferon therapy in children for 6 to 12 months leads to a complete response rate of 24 to 68% at the end of therapy, and a 36 to 56% of sustained response rate after a follow-up of 6 to 30 months. A similar response was only observed in 4% of the controlled group children. Combination therapy using ribavirin and interferon is effective in enhancing the rate of sustained response in adults. But whether combination therapy is more beneficial in children needs further confirmation.

Introduction

Hepatitis viruses can infect both adults and children. While most of the complications occur in adulthood, the infection may begin in infancy or childhood. Among viral hepatitis A to E, hepatitis B, C, and D may lead to chronicity. Chronic hepatitis D is very rare in children all over the world except in some endemic areas, while chronic infection of hepatitis B or C viruses can occur in children of any age, even during the perinatal period. Chronic infection of hepatitis B or C during childhood may cause chronic hepatitis, liver cirrhosis, and liver cancer during childhood or later in adulthood. Since carcinogenesis takes a certain period of time, hepatocellular carcinoma related to hepatitis virus infection will occur much earlier than those infected in adulthood.

Chronic hepatitis B virus infection

Hepatitis B virus (HBV) infection is a worldwide health problem. About two billion people in the world have been infected by HBV, and 350 million of them became chronic hepatitis B antigen carriers. In hyperendemic areas, while most of the complications of chronic HBV infection develop in adulthood, primary HBV infection occurs mainly during infancy or early childhood.

In areas with prevalent HBV infection, such as Asia, Africa, southern Europe and Latin America, primary HBV infection occurs mainly during infancy and childhood. The HBsAg carrier rate is approximately 2 to 7% in areas with intermediate prevalence and 7 to 20% in hyperendemic areas. In rural Senegal, 25% of children are infected by the age of 2 years; at age 15, the infection rate rises to 80%. In Taiwan, most chronic HBsAg carriers are infected before 2 years of age. The HBsAg seropositive rate is 5% in infants, and increases to 10% at 2 years of age. The HBsAg carrier rate remains stationary thereafter, while the infection rate reflected by anti-HBc seropositivity, reaches 50% by the age of 15 years.

Perinatal transmission from HBsAg carrier mothers to their infants is a very important route of transmission leading to chronicity. It accounts for the transmission route of 40-50% of HBsAg carriers in many hyperendemic areas. Around 90% of the infants of the HBeAg seropositive carrier mothers became HBsAg carriers, irrespective of a high or low HBsAg carrier rate in the population. Infection at an early age is an important factor determining the outcome of infection.

The two most important routes of horizontal transmission are highly infectious family members, such as siblings, and improperly sterilized syringes. Other sources of infections include institutionalized children, multiple or large amounts of blood transfusions, procedures piercing skin, etc.

Chronic HBV infection can occur in children of any age. In hyperendemic areas, it is not rare to see children suffering from acute or fulminat hepatitis B, acute exacerbation of chronic hepatitis B, and occasionally hepatitis B-related liver cirrhosis

or hepatocellular carcinoma. Understanding the epidemiology and natural course of HBV infection and the effect and current problems of hepatitis B immunoprophylaxis is crucial for the global control of HBV infection and its related liver diseases.

Immunoprophylaxis against HBV infection

Immunoprophylaxis is the most cost-effective way to achieve global control of HBV infection and its related complications. Passive immunization using hepatitis B immunoglobulin (HBIG) provides temporary immunity.

Infant of highly infectious mothers

To protect against HBV transmission from highly infectious mothers (HBeAg positive mothers), a combination of passive and active immunization will give the highest efficacy. Hepatitis B immunoglobulin (HBIG) should be given within 24 hours after birth. The first dose of hepatitis B vaccine should be given within the first week after birth. The second and third doses should be given one and six months later.

Infants of mothers with low or no infectivity, or in areas with limited financial resources

In areas where HBV infection prevalence is low, with limited financial resources, immunization with three doses of HBV vaccine in a 0,1 and 6 month(s) schedule without screening the maternal HBV markers is a reasonable strategy for the universal vaccination program. Infants of HBsAg negative mothers are recommended to receive three doses of HBV vaccine without HBIG.

Experience of pilot clinical trials for HBV vaccination

The HBV vaccine in clinical use can be divided into plasma and recombinant vaccines. The plasma vaccine is derived from plasma of HBV carriers. Recombinant HBV vaccine appeared on the market in the 1990s. Recombinant vaccines can be produced from yeast or other cells by expressing hepatitis B surface gene.

Before the era of HBV vaccination, passive immunization with three doses of hepatitis B immunoglobulin starting from within 24 hours after birth was useful in preventing perinatal HBV infection in high-risk infants (of HBeAg positive HBsAg carrier mothers)[1], reducing the HBsAg carrier rate from 90% to 23%, with an efficacy of around 75%. Active immunization with three or four doses of hepatitis B vaccine has proved to be immunogenic in more than 90% of neonates of non-carrier mothers or HBeAg-negative carrier mothers, and with around 75 to 78% efficacy in infants of high risk mothers. With combination of passive and active immunization, i.e. one dose of hepatitis B immunoglobulin within 24 hours after birth followed by three or four doses of hepatitis B vaccine, the carrier rate was reduced to 3% in pilot studies, and to 14% in the general population. The efficacy was increased to 85-95% in high-risk infants.[1 8]

Long term persistence of immunity

The immune response after vaccination can persist for more than 10 years, even after the level of anti-HBs becomes undetectable. Immunologic memory to HBV, most sensitively detected by IL-5 production of T cells remains intact.[2] None of the long-term followed vaccinees became chronic carriers, although approximately 1% of the vaccinees were infected and sero-converted to be anti-HBc positive.[3] A booster dose is thus not recommended within 10 years after the primary immunization.

The effect of universal hepatitis B immunization on the control of liver diseases in children

Decrease of total infection Rate and chronicity rate

Universal hepatitis B vaccination has effectively reduced the rate of chronic HBV inmfection in worldwide examples. The first universal hepatitis B vaccination program in the world was launched in Taiwan in July 1984.[4] The seroprevalence rates of HBsAg in Taiwan children before and 10 years after the installation of the vaccination program are shown in Table 1.[5-7]The HBsAg carrier rate decreased significantly from around 10% before to <1% after the vaccination program in children younger than 10 years old. This vaccination program has reduced both the perinatal and horizontal transmission of HBV.[5]

In reports from countries such as Gambia[6] and Italy,[7] hepatitis B carrier rates were reduced from 5 to 10% to less than 1%. Universal vaccination has a greater efficacy of HBV infection control than selective immunization.[7]

Decrease of the incidence of childhood hepatocellular carcinoma

After the launch of a universal hepatitis B vaccination program in July 1984 in Taiwan, we have successfully demonstrated the decline of the incidence of hepatocellular carcinoma in children. The annual incidence of hepatocellular carcinoma in children was reduced from 0.52 per 100,00 children born before July 1984 to 0.13 per 100,000 children born after July 1984.[8] A similar trend of decrease of childhood HCC has also been observed afterwards. Further decline of the incidence of hepatocellular carcinoma in adults is expected in the future long-term study.

Problems for the control of HBV infection

There are several problems we have to overcome to achieve the goal of eradicaiton of HBV infection and its related diseases (Table 2). The first problem is vaccine failure or non-responders. Intrauterine infection is a difficult problem to solve. Its rate was estimated to be less than 5% in infants of high risk (HBeAg seropositive) mothers. In a ten year follow-up study in Taiwan, 2.4% of infants of high risk mothers were persistently HBsAg seropositive since the first day of life in spite of the combination use of HBIG and HBV vaccine. The rate of hepatitis B surface mutant at the a determinant site, which is the target of the HBV neutralization antibody, increased from 7.8% before the vaccination program to 28.1% in Taiwanese HBsAg carrier children born after the universal vaccination. Because of its low prevalence, currently it is not considered a major problem for HBV immunophylaxis. But it may be a future problem, if the cumulative rate of hepatitis B surface mutants increases gradually.

Misinformation about the safety of HBV vaccination, though without solid basis, has led to an anti-vaccination stance in some people. Although without adequate evidence to support the causal association, reports of multiple sclerosis or other demyelinating diseases occuring in adults who had recently received hepatitis B vaccination have raised concerns about the safety of HBV vaccine. French authorities suspended the school-based adolescent hepatitis B vaccine programs in October 1998 while continuing the universal infant immunization program.

In addition, the relatively high cost of HBV vaccine for people in hyperendemic areas with very limited financial resources — making the universal vaccination program out of reach for people in those areas — needs to be addressed. Also, the mercury content, though within the safety limit, should be eliminated from the vaccine product.

Aims of antiviral therapy in children with chronic hepatitis virus infection

Since problems still remain even after HBV vaccination, children with chronic HBV infection are still at risk of HBV-related liver diseases and need to be properly treated. Current antiviral therapy for HBV infection is focused mainly on the diseases in adulthood, because of the safety concern and also the high viral replication in children. However, it is very important, though difficult, to shorten the duration of hepatic inflammation and to eradicate the virus as early as possible. This is true particularly for a virus like HBV, with a possibility of integration of the viral genome into the host genome and carcinogenesis.

The current antiviral therapy is not able to reach the goal of viral eradication. Inhibition of viral replication has become the more appropriate aim of antiviral therapy at present. In addition to viral inhibition, prevention of liver damage and its related complication is also the goal of current antiviral therapy for chronic HBV infection.

Treatment of chronic HBV infection

Interferon therapy

Information concerning interferon therapy in children with chronic hepatitis B virus is limited by the small number of children who received interferon therapy and the control group. As shown in Table 3, interferon therapy is not effective for HBe seroconversion in Asian carrier children with normal liver enzymes. In those with elevated aminotransferases, the data are similar to that in adults. The rate of HBe seroconversion and normalization of aminotransferase levels were 20-40% higher in the treatment group than the control group. The data from single a study are usually not statistically significantly beneficial in the interferon therapy group. However, meta-analysis by summation of the reported worldwide data shows significant effect of interferon therapy in children mostly from the Western world. However, the effect of interferon therapy effect in the Asian children in whom more perinatal or early infection occurs should be further evaluated.

Lamivudine Therapy

Lamivudine is a 2‘,3’-dideoxynucleoside which is phosphorylated by intracellular enzymes to form lamivudine triphosphate. Lamivudine triphosphate can inhibit DNA synthesis by terminating the nascent proviral DNA chain and interfere the reverse-transcriptase activity of human immunodeficiency virus (HIV) and hepatitis B virus (HBV). After oral administration, lamivudine is well absorbed and has good bioavailability (mean absolute bioavailability of >80% in adults and 68% in infants and children).[9] In the clinical trials in adults, lamivudine rapidly reduces HBV DNA to undetectable levels in a daily dose of 100 mg or more.

Reponse to lamivudine therapy

A complete response was defined as disappearance of HBeAg and apppearance of anti-HBe, disappearance of HBV DNA, and normalization of aminotransferase. Two-year lamivudine therapy in adults is associated with a 27% sustained anti-HBe response, while one-year therapy is associated with a 17% sustained anti-HBe response.[10]

Experience in children

The experience of lamivudine in treating chidlren with chronic hepatitis B is very limited. Lamivudine was well tolerated in the 53 treated European and Canadian children age 2 to 17 years.[11] Around one-fifth of patients who received one month of lamivudine therapy experienced drug-related adverse events. The most commmon adverse events were ear, nose and throat problems, gastrointestinal symptoms, general symptoms (malaise and fatigue), and lower respiratory symptoms. No serious side effect or withdrawal occurred during therapy or follow up.

Lamivudine was reported to negatize or reduce most of HBV DNA in pediatric patients after one month of therapy in a multicentered study in European and Canadian children. A dramatic fall of HBV DNA of 99.9% was noted. However, HBV DNA returned to the pretreatment levels in all the patients after cessation of lamivudine. HBeAg loss rate remained low after three months of follow up. Further prolonged treatment is ongoing to obtain significant e seroconversion rate and avoid viral relapse after therapy cessation.

Lamivudine 100 mg daily is the recommended dose for adults. The recommended dose for children is 3 mg/kg oral use daily.[11] Children who were HBsAg seropositive for more than six months and with positive HBV DNA and elevated aminotransferase levels for more than three months are the candidates for lamivudine therapy.

Special conditions and compassionate therapy

For hepatitis B carrier children who will receive transplantation or immunosuppressants, children with repeated acute exacerbation of liver function, or children with fibrosing cholestatic hepatitis, lamivudine therapy is helpful in reducing HBV DNA, decreasing the hepatic inflammation and permanent injury, and even prevent death from liver failure.

Monitoring response to therapy

In order to ensure the effect of therapy, the drug compliance should be monitored carefully, particularly in adolescents.

Monitoring the changes of laboratory data including serum HBV DNA by hybridization method, liver function profiles, complete blood cell counts, amylase, lipase, urea nitrogen, and creatinine are also very imporatant to evaluate the effect and adverse reactions of the therapy.

Liver histologic changes before and one year after therapy provide very important information about the benefit of lamivudine therapy in preventing liver damage and viral clearance in the liver.

Cessation of treatment

Lamivudine will be stopped if any related adverse reaction developed. After completion of one or two years of lamivudine therapy, cessation of lamivudine may be considered. However, replication of HBV may resume after stopping lamivudine. Therefore HBV DNA and hepatitis B e antigen and antibody should be carefully monitored to observe the rate of sustained negative HBV DNA and positive anti-HBe. Liver function and coagulation profiles also should be monitored to observe any exacerbation of liver function.

Problems during or after therapy

Information on lamivudine-resistant strains and its clinical relevance are still lacking in children. Since the replication of lamivudine associated HBV mutant strain was reported to be less competent than the wild type, and no association of a decrease of liver histologic response to lamivudine was noted with mutant strains in adults at one year of therapy, interrupting lamivudine therapy does not seem indicated in children upon the detection of mutant strains.

Chronic hepatitis C virus infection in children

Epidemiology

Prevalence of HCV infection

The seroprevalence of hepatitis C virus infection in adults is around 0.8 to 1.5% in the adult population in most parts of the world, except some hyperendemic areas. In children, the seroprevalence rate is much lower (mostly 0 to 0.2%) than that in adults. HCV infection occurs mainly in high risk children. It is more difficult to conduct studies in children than adults because of its low prevalence. The majority of studies in children have been conducted in high risk children, such as children who have been exposed to blood products (children with hemophilia, thalassemia, blood transfusion, hemodialysis, malignancy, etc.) or children of HCV infected mothers.

Route of transmission

Mother-to-infant transmission

Compared with HBV infection, the level of HCV virus in circulation is much lower. Infants of HCV infected mothers are at risk of being infected by their mothers during the perinatal period. Fortunately, the mother-to-infant transmission rate of HCV is much lower than that in HBV. The level of maternal HCV RNA is the most important determinant of the outcome of the infants. In infants of mothers who are HCV RNA seropositive and HIV seronegative, the rate of maternal transmission were reported to be 0 to 15%, with a mean rate of 4.7%.

Parenteral cxposure to HCV

Transfusion is the most important route of HCV transmission in children. Prospectively studying the children who received open heart surgery and blood transfusion in Taiwan revealed that the transmission rate of HCV infection was around 5%, which is lower than that in adults (8.8 to 12.5%). Screening of anti-HCV for the donated blood has prevented most of the post-transfusion HCV infection.

Thalassemic children who received multiple transfusion are another high risk group. The HCV infection rate in thalassemic children ranged from 43% to 60%.

Among the HCV infected thalassemic patients, more than 90% of them had elevated alanine aminotransferase (ALT) during follow-up, while only around one third of the HCV-uninfected thalassemic patients had elevated ALT. Quantitation of serum HCV RNA in thalassemic patients showed mild fluctuation between $1x10^6$ to $5x10^8$ copies/ml.

Approximately 15 to 20% of the pediatric hemodialysis populations and 3 to 4% of survivors of childhood malignancy were infected by HCV.

Sporadic Cases

The proportion of cases without identifiable risk factor has consistently ranged from 35 to 40%. Intramuscular injection using improperly sterilized syringes or needles is the most likely route of transmission.

Natural vourse of HCV infection in children

After primary infection with HCV, 60 to 80% of children ran a chronic course. HCV RNA is detectable in serum by two weeks after exposure, and anti-HCV is detectable in serum by four to eight weeks. Most chronically infected children remain asymptomatic. The liver function profiles are usually normal in children after HCV infection. However, transient or persistent elevation of aminotransferase levels is not uncommon. It occurs in most of the reported cases through mother-to-infant transmission, although a number of children were reported to clear serum HCV RNA spontaneously. Histologic studies of the liver in chronically infected children is limited. Chronic persistent hepatitis or minimal histologic changes are the main findings.

Prevention of HCV infection

Although immunization reduces the infection rate and carrier rate of hepatitis B, currently no vaccine is available for the prophylaxis of hepatitis C virus infection. Screening of the blood and use of disposable syringes and needles have reduced post-transfusion HCV infection and hepatitis effectively. In our series, post-transfusion HCV infection occurs in 5% of children who received blood transfusion for open heart surgery. The incidence declined to 0% after screening of blood since 1992. Educating the public to avoid improperly sterilized invasive procedures is also very important.

Treatment of chronic HCV infection

Interferon therapy

Experience in Adults

The Natonal Institutes of Health, USA consensus for the inital treatment of patients with chronic hepatitis C recommended that the duration of treatment is 12 months and the dosage is 3 MU three times a week. A sustained response to interferon therapy with disappearance of HCV RNA and normalization of aminotransferase is approximately 15 to 25% after 48 weeks of therapy.

Experience in children

There have been several studies of interferon therapy in children with chronic hepatitis C (Table 4). However, the number of cases were small and very few had controlled studies. Interferon therapy in children for 6 to 12 months leads to a complete response rate of 24 to 68% at the end of therapy, and a 36 to 56% of sustained response rate after a follow-up of 6 to 30 months. A similar response was only observed in 4% of the controlled group children.

Combination therapy with ribavirin and interferon

Ribavirin is a synthetic guanosine analog with intracellular virustatic effect. It is rapidly absorbed through oral use. Its combination with interferon revealed an enhanced rate of sustained response. A sustained response rate of 31% to 35% after 24 weeks of combination therapy, and 38% to 43% after 48 weeks of combination therapy was observed.

Future Prospects

The best way to control hepatitis virus infection is prevention. The World Health Organization has previously recommended that universal hepatitis B immunization be introduced in all countries by the end of 1997.[15] Up to now, a total of approximately 100 countries have followed this recommendation. With the integration of the hepatitis B vaccination program into the Expanded Immunization Progaram in most countries in the world, chronic hepatitis B virus infection will be further reduced in the next century. The main problems for hepatitis B control in the future will be vaccine failure and escape from the vaccination program. Further understanding of the immune defects and pathogenetic mechanisam of chronic hepatitis B virus infection will enhance the development of better therapeutic and preventive agents and vaccines focused on the T cell-epitopes of hepatitis B virus, which can be recognized by convalescent patients.

Chronic hepatitis C virus infection is also decreasing after blood product screening and usage of disposable syringes, needles and sterilization of other invasive instruments. The development of HCV vaccine is still unsuccessful due to the high genetic mutation rate of this RNA virus. Further prevention and reduction of hepatitis C virus infection depends on the future success of the development of hepatitis C vaccine and the new antiviral strategies.

A new treatment strategy of antiviral therapy is combination therapy as used in anti-HIV or anti-HCV therapy. Experimental studies in new antiviral agents, such as nucleotide analogs, antisense oligonucleotides, DNA vaccines, etc. have potential (Table 5), but more data in efficacy and safety are needed to improve the effect of current antiviral therapy for chronic hepatitis B or C virus infection and their related complications .

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Table 1

Seroprevalence of Hepatitis B Surface Antigen Before and After Universal Vaccination Against Hepatitis B Virus

Taiwan			Italy			Gambia		
Age (Yr)	1984 (%)	1994 (%)	Age (Yr)	1978 (%)	1993 (%)	Age (Yr)	No (%)	Yes (%)
<1	5.1	0.0	0-5	8.6	1.0	4	9.56	0.55
1-2	10.7	0.5	6-12	9.3	0.5			
3-4	10.1	0.3	13-60	14.6	4.0			
5-6	10.6	0.8						
7-8	9.7	0.9						
9-10	11.0	1.5						
11-12	9.1	6.8						

Table 2

Current Problems of Hepatitis B Vaccination

1. Vaccine failure or unresponsiveness
   a. Intrauterine infection or high maternal viral load
   b. Immune compromised host
   c. Genetic unresponsiveness
   d. Vaccine escape mutant
2. Misinformation about safety of vaccine or ignorance
3. Relatively high cost
4. Competition from other vaccines

Table 3

The Effect of Interferon Therapy on Hepatitis B Virus Infection in Children

		Rate of HbeAg Seroclearance	
Place and Authors	ALT at Therapy	Control Group	IFN Therapy Group
Europe			
Ruiz-Moreno, 1995	45>IU/L	—	34% (17/50)
Barbera, 1994	no limit	14% (5/37)	26% (10/39)
Vajro, 1996	>1.5X of nl	11% (1/9)	48% (10/21)
Gregorio, 1996	no limit	13% (4/31)	38% (24/64)
Sokal, 1998	>2X of nl	11% (8/74)	26% (18/70)
America			
Narkewicz, 1995	>2X of nl	—	78% (7/9)
Asia			
Lai, 1991	no limit	0% (0/30)	8% (5/60)
Chang, 1997	>80 IU/L	46% (6/13)	73% (8/11)
Meta-analysis			
Toree, 1996	no limit	11% (12/113)	23% (29/126)*

P=0.026

Table 4

Complete Response (Negative HCV RNA and Normal Aminotransferase) Rate to Interferon Therapy in Children with Chronic Hepatitis C

	Control	Rx Duration	Rx Response	FU Duration	FU Response
Ruiz-Moreno	—	3MU/m2@,6M	36%(4/11)	24M	45%(5/11)
Bortolotti	0%(0/13)	5MU/m2@,4-12M	71%(10/14)	24M	54%(7/13)
Iorio	10%(1/10)	3MU/m2@,12M	45%(5/11)	30M	45%(5/11)
Jonas	—	3MU/m2@,12M	24%(5/21)	—	—
Fujisawa	—	0.1MU/Kg/d,6M	33%(14/32)	?	?
Masuoka	—	0.1MU/Kg/d,6M	68%(15/22)	12M	36%(8/22)
Sawada	—	0.1MU/Kg/d,6M	50%(12/24)	18M	56%(10/18)

Table 5

Current and Future Strategies for the Therapy of Chronic Hepatitis

I. Interferon: antiviral and immunomodulatory agent

II. Antiviral therapy
   1. Nucleoside analog
   2. Antisense oligonucleotide
   3. Gene therapy

III. Immune modulatory therapy
   1. Thymosine
   2. DNA vaccine

IV. Combination therapy

Notes

A look to the future? Gene therapy for gastrointestinal and hepatic diseases

Lynn Jorde, PhD

LEARNING OBJECTIVES

At the conclusion of this session, participants will:

1. Understand the basic methods involved in somatic and germline gene therapy;

2. Be familiar with the advantages and limitations of various types of gene therapy vectors (e.g., retroviruses, adenoviruses, adeno-associated viruses, liposomes);

3. Become aware of the potential, as well as the limitations, of gene therapy for inherited and non-inherited disorders and, in particular, for gastroin testinal and hepatic disorders.

Gene Therapy for Gastrointestinal and Hepatic Diseases

Lynn B. Jorde, PhD

I. Introduction and basic definitions

A. Current status: several thousand patients are enrolled in more than 400 gene therapy protocols

B. Somatic vs. germline therapy

1. Somatic replacement therapy: useful for many loss-of-function mutations like cystic fibrosis, ADA deficiency, hemophilia A and B

2. Somatic gene blocking therapy: potentially useful for gain-of-function mutations like Huntington disease

C. Ex vivo vs. in vivo gene insertion

D. Types of cells best suited to transfection

II. Major gene therapy delivery vectors

A. Retroviruses are the most commonly used vectors

1. Advantages

a) Relatively stable and efficient integration

b) Insertion directly into the genome means that retrovirally inserted genes will be replicated as cells divide

c) Biology of retroviruses is well understood

2. Disadvantages

a) Limitations on the size of the insert (about 8 kb)

b) Random integration into the genome could result in mutations or carcinogenesis

c) Most retroviruses (with the important exception of lentiviruses) will integrate only into actively dividing cells

3. Disease example: severe combined immune deficiency due to adenosine deaminase deficiency

B. Adenoviruses

1. Advantages

a) Major advantage over retroviruses is that adenoviruses will insert into nondividing cells

b) Capable of receiving larger inserts than retroviruses (about 30 kb)

c) Lack of integration directly into host DNA reduces potential for insertional mutagenesis or activation of proto-oncogenes

2. Disadvantages

a) Lack of integration directly into host DNA means that these inserted genes are not replicated as cells divide; vectors must be re-inserted multiple times for most diseases

b) Not all of the adenoviral genome is removed; this combined with repeated introduction often results in an immune response

3. Disease example: cystic fibrosis

C. Adeno-associated viruses

1. Advantages

a) Stable integration into host DNA

b) Can integrate into non-dividing cells

c) No known association with human disease

2. Disadvantages

a) Limited insert size (about 5 kb)

b) Biology less well understood than that of retroviruses

D. Liposomes: an example of a non-viral vector

1. Advantages

a) No immune response

b) Capable of accepting large DNA inserts

2. Disadvantages

a) Low efficiency of transfer into cells

III. Challenges facing gene replacement therapy

A. Transient and low-level expression of the gene product

B. Difficulties in properly regulating gene product expression (e.g., over-expression of globin is as hazardous as under-expression)

C. Difficulties in reaching target tissue

IV. Somatic gene-blocking therapy

A. Antisense therapy: an oligonucleotide is synthesized that will hybridize with and block translation of the mRNA sequence of a gain-of-function mutation

B. Ribozyme therapy: enzymatic RNA molecules are synthesized that can cleave specific mRNA sequences, preventing them from being translated

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2. Disadvantages

a) Lack of integration directly into host DNA means that these inserted genes are not replicated as cells divide; vectors must be re-inserted multiple times for most diseases

b) Not all of the adenoviral genome is removed; this combined with repeated introduction often results in an immune response

3. Disease example: cystic fibrosis

C. Adeno-associated viruses

1. Advantages

a) Stable integration into host DNA

b) Can integrate into non-dividing cells

c) No known association with human disease

2. Disadvantages

a) Limited insert size (about 5 kb)

b) Biology less well understood than that of retroviruses

D. Liposomes: an example of a non-viral vector

1. Advantages

a) No immune response

b) Capable of accepting large DNA inserts

2. Disadvantages

a) Low efficiency of transfer into cells

III. Challenges facing gene replacement therapy

A. Transient and low-level expression of the gene product

B. Difficulties in properly regulating gene product expression (e.g., over-expression of globin is as hazardous as under-expression)

C. Difficulties in reaching target tissue

IV. Somatic gene-blocking therapy

A. Antisense therapy: an oligonucleotide is synthesized that will hybridize with and block translation of the mRNA sequence of a gain-of-function mutation

B. Ribozyme therapy: enzymatic RNA molecules are synthesized that can cleave specific mRNA sequences, preventing them from being translated

V. Gene therapy for nonhereditary diseases: examples

Notes

IBD Therapy in the New Millennium

James Markowitz, MD

LEARNING OBJECTIVES

At the conclusion of this session, participants will gain an understanding of:

1. Newer therapeutic modalities for Crohn's Disease and Ulcerative Colitis;
2. The data from Pediatric IBD Clinical Trials for the newest treatment for IBD;
3. New therapeutic approaches which will form the basis for future treatment for children with IBD.

IBD Therapy in the New Millennium

James Markowitz, MD

Traditional IBD Therapies

Anti-Inflammatories
- 5-ASA
- Corticosteroids

Nonspecific Immunomodulators
- 6-mercaptopurine
- Azathioprine
- Methotrexate

Newer "Semiselective" Immunomodulators

Cyclosporine/tacrolimus - calcineurin phosphatase inhibitors; suppress gene transcription of lymphokines, especially IL2

Þ Cyclosporine in Fulminant UC

	Adults	Children
Prevents imminent colectomy	75-80%	20-78%
Induces long term remission	43-55%	0-30%**

**Up to 67% if CSA given with 6MP + steroid

Þ Cyclosporine in Children with Active Crohn's Disease
- Initial Rx = iv bolus bid (4 mg/kg/day), then po CSA + 6MP
- 7/10 with clinical response within 2 weeks
- 6/10 ultimately required surgery by 6 months[1]

Þ Tacrolimus in Pediatric IBD
DDW 1997
- Severe colitis (4 UC, 2 Crohn's)
- Oral tacrolimus (0.1 mg/kg/dose q12h) x 90 days; 6MP started after 45 days
- 5/6 improved and avoided surgery
- Hospital discharge on full oral diet 10-14 days after starting tacrolimus
- 3/5 responders weaned successfully to 6MP[2]

Mycophenolate mofetil - inhibitor of *de novo* purine synthesis; suppresses both T and B lymphocyte proliferation

Þ Clinical Trials: Response to MMF

1. Graz, Austria: 6 AZA intolerant CD patients
   - 4 active PD, 2 steroid dependent, active inflammatory CD
   - All with decreased CDAI over 6 months[3]
2. Los Angeles: 11 6MP/AZA resistant CD patients
   - 2 partial + 1 complete response
   - 4 intolerant + 4 no response[4]
3. Lubeck, Germany: 24 chronic active CD pts
   - 10/24 remission after 3 months
   - All but 1 relapsed after 6 months[5]
4. Graz, Austria: 20 AZA intolerant or unresponsive CD pts
   - 12/20 clinical response within 6 months
   - 8/12 responders relapse within 3 yrs[6]

Targets for IBD Therapies in the New Millennium

Selective Immunomodulation

Cytokines

Leukocyte-Endothelial Interactions

Bacterial Flora

Pathogens

Endogenous populations

Gut Nutrition/Metabolism

New Technologies Provide Potential Approaches for Novel Therapeutics

Monoclonal antibodies - neutralize target antigen (eg cytokine) by binding to free and/or membrane bound target

Antiinflammatory cytokines - endogenous cytokines capable of downregulating activity of proinflammatory cytokines

Antisense oligonucleotides - prevent gene expression by binding to mRNA of specific gene preventing translation and gene product formation

Receptor antagonists - compound capable of binding to target receptor without activating receptor activity

Monoclonal Antibodies

Targets

Þ TNFa (Infliximab, CDP571)

Þ IL 12

Þ Integrins
- a4 (Natalizumab)
- a4b2 (LDP-02)

Infliximab: Chimeric anti-TNF monoclonal antibody

Þ Adult data - Active inflammatory CD
- 65% clinical response, 33% clinical remission 4 weeks after single infusion (5 mg/kg)[7]
- Among those responding to initial infusion, 90%

maintained response to 44 weeks with 4 subsequent infusions given at 8 week intervals; 35% of those who received only a single infusion also remained well 44 weeks after initial treatment[8]

- Adult data - Fistulae:
  - ~55% of patients have closure of at least half of their fistulae after 3 infusions over a 6 week period; relapse common after infusions discontinued[9]
- Pediatric Data
  - 65 - 93% pts with active inflammatory CD respond within 4 weeks of infusion, with significant decreases in disease activity scores (PCDAI or Harvey-Bradshaw) and ESR
  - 25 - 74% remission by 8-10 weeks
  - Closure of fistulae possible
  - Minimal infusion reactions, respond to diphenhydramine
  - Effects often wane by 8 - 12 weeks
  - ? more prolonged effect in children with <2 yrs CD[10-14]
- Adverse reactions: Infusion reactions, abscess formation, SLE-like reaction, bowel obstruction, ?lymphoma

CDP571: "Humanized" anti-TNF monoclonal antibody

- Randomized controlled trial: 169 adults with active CD
  - 54% (vs 27% in placebo) improved at 2 weeks
  - ~50% fistulas closed
  - Improvement maintained with repeat dosing q12 weeks
  - No infusion reactions, drug induced lupus, lymphoma[15]
- Randomized controlled trial: 71 adults with steroid dependent CD
  - Facilitates withdrawal of steroids[16]

New anti-TNF therapies

- **Thalidomide**
  - Decreases TNFa production by accelerating the degradation of the mRNA encoding for the protein
  - 58-100% response, 17-25% remission at 4 weeks
  - Allows steroid taper[17,18]
- **Etanercept** (TNF receptor fusion protein)
  - Inactivates TNF by binding to receptor binding site of TNF
  - 60% active CD pts decreased CDAI at least 70 points by week 2[19]
- **Active anti-TNF immunization** (preclinical - mice)
  - Inserting foreign T cell epitopes into TNF results in autoantibodies which cross react with native TNF and interfere with TNF-receptor interactions[20]

Antiinflammatory Cytokines

- IL 10
- IL 11

IL-10

- Down-regulates production of proinflammatory cytokines (IL-1, IL-2, TNF)
- Intestinal histopathology of IL-10 gene knockout mice resembles Crohn's disease
- Serum IL-10 is elevated in active UC and CD
- ? IL-10 = naturally occurring damper of the acute inflammatory response in IBD

Recombinant human IL-10 (rhu IL-10): Clinical Trials

- Multicenter, double blind, dosing trial
- Adults with steroid refractory CD (CDAI >200):

	rhu IL-10 (n=32)	Placebo (n=13)
Remission	50%	23%
Response	30%	22%[21]

- rhu IL-10 causes dramatic decrease in histologic inflammation after 4 weeks of Rx[22]

Recombinant human IL11 (rhu IL-11)

- Multicenter, placebo-controlled dose-ranging trial
  - 76 adults with active CD
  - CD activity decreased in 33-42% vs 7% controls
  - Side effect: thrombocytosis[23]
- Preclinical animal studies demonstrate decreased colitis with oral and enema preparations of rhu IL-11[24]

Antisense Oligonucleotides (AO)

Targets

- NF kB
- ICAM-1 (Isis 2302)
- TNFa (Isis 25302)
- **NF-kB antisense oligonucleotide**
  - NF-kB: a family of transcription factors for lymphocytes and macrophages which regulate the promoters of a variety of genes controlling the production of cytokines, as well as adhesion molecules and other acute phase reactants
  - p65 subunit overexpressed in:
    - murine TNBS colitis
    - IL-10 knockout mouse
    - Crohn's disease
  - AO directed at the promoter region of the p65 subunit of NF-kB:
    - abrogate clinical & histological experimental murine colitis

» suppress IL-1, IL-6, TNF-a[25]

- **ICAM-1 Antisense oligonucleotide (ISIS-2302)**
  - Preliminary controlled trial:
    - » 20 pts active steroid refractory CD
    - » Remission: 47% vs 20% after 1 month
    - » At 6 months, 5/7 remitters still in remission[26]
  - Placebo controlled, dose ranging trial:
    - » 77 pts with steroid refractory CD
    - » Effect no better than placebo[27]

New Therapies Directed at Leukocyte-Endothelial Adhesion

- **Antisense oligonucleotides against ICAM-1 and VCAM-1[28]**
- LDP-02 (humanized anti-lymphocyte adhesion molecule monoclonal antibody)[29]
- Bosentan (endothelin receptor antagonist)[30]
- G1270384X (inhibitor of ICAM-1 and E-selectin expression[31]

Receptor Antagonists

Targets

- IL1 receptor (IL1RA)
- Endothelin receptor (Bosentan)

Gut Flora in IBD

Crohn's disease appears to be associated with:

- Increased intestinal permeability
- Serologic response to intestinal pathogens and non-pathogens
- Loss of immunologic tolerance to enteric microflora

Gut Flora and Animal Models of IBD

- Animal models of IBD (eg. IL-10 knockout mice) do not develop inflammation if raised in a germ free environment
- In normal environment, development of inflammation in IL-10 KO associated with loss of enteric lactobacilli
- Supplementation of lactobacilli to IL-10 KO prevents development of inflammation[32,33]

- Probiotics in IBD
  - Healthy volunteers
    - » L.GG decreases T cell response to foreign intestinal flora
    - » Increases IL-10 and decreases TNFa production[34]
  - Active CD
    - » L. salivarius decreases TNFa but no change in other cytokines[35]
  - Prevent CD relapse
    - » 5-ASA: 6/16 relapse at 6 months
    - » S. boulardii + 5-ASA: 1/16 relapse[36]
  - Prevent and treat pouchitis
    - » 2/20 probiotic treated vs 8/20 placebo controls with acute pouchitis by 1 yr after IPAA[37]
    - » L. GG + fructooligosaccharide suppressed symptoms and endoscopic changes in 10 pts with antibiotic refractory or dependent pouchitis[38]

Antibiotics in IBD: Role of Mycobacteria

- Role of mycobacteria as etiologic agents for Crohn's disease remains controversial
- Multiple studies of anti-mycobacterial therpies in IBD demonstrate improved clinical status of patients
- Mechanism of action unclear[39,40]

Nutritional Therapy

Nutrition as Primary Therapy: Crohn's Disease

- Overall, meta-analysis demonstrates odds ratio favoring steroids over enteral feeding for inducing remission in active disease
- In pediatrics, remission rates similar with steroids or enteral feeds[41]
- Amino acid based feed not superior to intact protein formula[42]
- Glutamine enriched formula no different than low glutamine diet[43]

Novel Nutrition Based Investigations: Crohn's Disease

- Low LCT diet important ?? Studies conflict[44,45]
- Growth factor supplementation
  - CT3211 (Nestle) - Specific polymeric diet rich in transforming growth factor b2
  - 23/29 (79%) children with active CD in remission after 8 weeks[46]

Nutrition as Primary Therapy: UC

- No conclusive data demonstrates clinical response to nutritional therapy in inducing or maintaining remission in UC

Novel Nutrition Based Treatments for UC

- w-3 Fatty acids
  - Supplement (5.6 gm/day) in mildly active UC improved clinical and histology score compared to placebo[47]
- UCF (Ross)
  - Nutritionally complete formula supplemented with fish oil, fructooligosaccharides, gum arabic, antioxidants
  - Mild to moderate adults with UC
  - UCF group: Similar reduction of disease activity, more rapid weaning of steroid compared to placebo[48]

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Portal hypertension: New approaches

Deirdre A. Kelly, MD

LEARNING OBJECTIVES

As a result of this session, participants will learn that:

1. Portal hypertension in children is a potentially life threatening complication of either extrahepatic portal vein thrombosis or hepatic fibrosis or cirrhosis;

2. Medical management includes acute recuscitation for variceal bleeding, endoscopic band ligation or radiological insertion of TIPSS;

3. The role of portosystemic shunting has reduced following effective medical management but is indicated for recurrent variceal bleeding in children with EHPVO, while liver transplantation is more appropriate for children with end stage liver failure.

Portal Hypertension: Pharmacologic, Radiologic, Endoscopic and Operative Approaches

Deirdre A. Kelly, MD

Definition

Portal hypertension is defined as a portal venous pressure of >11 mm of mercury or a splenic pulp pressure of >16mm of mercury.

The main causes in childhood of portal hypertension are extra hepatic portal vein obstruction (EHPVO); non cirrhotic portal hypertension (NPH); hepatic fibrosis or cirrhosis, occlusion of the hepatic veins (Budd-Chiari syndrome) or secondary to a hepato-portal arterio-venous fistula (HAF).

Mortality is related to death from gastrointestinal haemorrhage, which is approximately 5-9% in children with extra hepatic portal vein obstruction and was higher in children with cirrhosis prior to the successful development of liver transplantation.

Clinical Features

The clinical features of portal hypertension vary according to aetiology. In children with extra hepatic portal vein obstruction or non cirrhotic portal hypertension, children may present with asymptomatic splenomegaly, hypersplenism, anaemia, growth failure or ascites. Children with portal hypertension secondary to liver disease will present with the features of chronic liver disease such as jaundice, malnutrition, ascites and other hepatic complications. All children with portal hypertension, irrespective of aetiology, may present with haematemesis and melaena.

Diagnosis

The diagnosis of portal hypertension may be made clinically by any of the above features and is confirmed radiologically by using doppler ultrasound. Ultrasound may demonstrate splenic or gastric varices and provide information about the size of the portal vein and the direction of portal flow which may be retrograde in severe portal hypertension.

Other radiological measures of value in the diagnosis of portal hypertension are hepatic angiography and venography which may be combined with measurement of portal pressures. The recent development of magnetic resonance imaging (MRI) has dramatically improved the ability to diagnose portal hypertension with good quality imaging of hepatic vasculature.

Further confirmation of portal hypertension is obtained by endoscopic gastroscopy to demonstrate oesophageal and gastric varices and the presence of portal gastropathy.

Extra hepatic portal vein obstruction (EHPVO)

EHPVO is a significant cause of portal hypertension in children, accounting for 25-40% of children with variceal bleeding. The aetiology is usually unknown although umbilical vein catheterisation or sepsis and coagulation disorders such as protein C and protein S deficiency have been implicated.1 Most children will have a portal vein cavernoma which will be identifiable on doppler ultrasound and confirmed by angiography.

The extent of the portal vein obstruction varies in degree. There may be occlusion of the main portal vein with or without intrahepatic vein involvement, while the thrombosis may extend to the mesenteric and splenic veins particularly in children with coagulation disorders.[2]

Other anomalies such as cardiovascular disease, duodenal atresia or congenital hepatic fibrosis have been reported in association with EHPVO.

Non cirrhotic portal hypertension (NPH)

This rare condition accounts for approximately 40% of portal hypertension in India. The portal vein is patent and the liver architecture is normal but liver histology demonstrates pre-sinusoidal venous obstruction which is associated with sub endothelial thickening of intra hepatic venous radicals. The diagnosis may be confirmed by hepatic angiography which demonstrates intra hepatic portal vein obstruction.[3]

Budd-Chiari syndrome

Budd-Chiari is a very rare cause of portal hypertension in children. The occlusion of the hepatic veins may be due to a congenital webb in the inferior vena cava, obstruction of the main hepatic vein by thrombosis secondary to a coagulation disorder or myeloproliferative disease.[4]

Hepatoportal arteriovenous fistula (HAF)

Congenital hepato portal arteriovenous fistula (HAF) is usually secondary to a congenital arterio-venous malformation of the liver but is occasionally acquired following surgery.

Ascites is a common presentation with a bruise over the liver. Portal hypertension may be severe and associated with congestion of the small intestine leading to malabsorption, rectal bleeding and failure to thrive.

The treatment of HAF is difficult and includes arterial embolisation, localised hepatic resection, ligation of the hepatic artery and occasionally, liver transplantation.[5]

Management of acute variceal haemorrhage

The management of acute variceal haemorrhage is similar for all forms of portal hypertension (Figure 1). The basic principles are: to assess the site and extent of the bleed, to resuscitate with albumin, fresh frozen plasma and red cell transfusions. H2 blockers such as ranitidine or proton pump inhibitors such as omeprazole, are commonly used although the efficacy is unknown.

Intravenous octreotide (3-5 mg/kg/hour) and/or glypression (0.3-1 unit/kg/hour) or vasopression (0.2-0.4 units/kg/hour) are first line therapy in order to reduce portal pressure.

Once the patient has been stabilised, it is essential to confirm the diagnosis by endoscopy and to carry out therapeutic sclerotherapy or band ligation as required. If bleeding continues despite sclerotherapy or band ligation, balloon tamponade with the modified Sengstaken Blakemore tube may be effective.

Endoscopic sclerotherapy

There are now a number of large series reporting the results of endoscopic sclerotherapy,[6-8] demonstrating successful variceal ablation from 84-96% with re-bleeding rates of 4-45% (Table 1, not included). There are significant complications which include oesophageal ulceration (20%), stricture (16%) and recurrent varices (8%). Endoscopic sclerotherapy is effective long term in obliterating varices but is not effective for gastric varices or hypersplenism.

Band ligation

Injection sclerotherapy has largely been replaced in adults by band ligation.[9] There are few reported studies in children but preliminary results indicate that between 70-100% of varices are ablated with re-bleeding rates of 15-30% and a lower complication rate although post therapy dysphagia is more common.[10]

Transjugular intrahepatic porto-systemic stent shunt (TIPSS)

In a small number of children with intractable variceal bleeding not controlled by conventional methods, bleeding may be controlled by insertion of a TIPSS.[11] This radiological technique involves identification of the anatomy of the portal vein by hepatic and mesenteric angiography, cannulation of the right internal jugular vein and introduction of the cannula and biopsy needle into the right hepatic vein. A guidewire is passed through a needle into the main portal vein and a stent (usually a metal prosthesis) is placed between the hepatic vein and the main portal vein.

A number of small studies reporting the use of TIPSS in children have indicated success rates of 80-100%. The success of the procedure is limited by the age and size of the child, the size of the portal vein (should be >2mm in diameter) and by the size of the stents (5-6mm in diameter). It is important that the stent is long enough to allow for growth of the child. Complications include occlusion of the stent, infection, abscess formation around the stent and development of encephalopathy.

The use of TIPSS to control portal hypertension in children with compensated liver disease, such as cystic fibrosis, is an increasing indication for this procedure.[12]

Porto-Systemic Shunt Procedures

The successful development of endoscopic sclerotherapy and band ligation in the treatment of portal hypertension has reduced the indications and necessity for porto-systemic shunt procedures. The most commonly used operations in children historically have been the mesocaval shunt and the distal spleno-renal shunt (Table 2).[13-15] More recently the meso-portal shunt has been described in which the extra hepatic portal obstruction is directly bypassed using a graft from the superior mesenteric vein to the left intrahepatic portal vein. This technique is only possible if the left intrahepatic portal vein is patent and it is necessary to demonstrate this prior to surgery using the combination of hepatic angiography and retrograde hepatic venography. The technique provides effective portal decompression, restoration of hepatic portal flow, reduction in splenomegaly and hypersplenism and resumption of normal growth.

The indications for systemic shunts include intractable variceal bleeding, severe hypersplenism or gastric varices. In some children with severe growth failure porto-systemic shunting restores normal growth presumably because of the circulation of growth factors. Occasionally it may be more appropriate to carry out a porto-systemic shunt to reduce the risk of variceal bleeding in children living far from a treatment centre for variceal haemorrhage.

In general, porto-systemic shunts are not performed in children who are considered for liver transplantation except as an emergency measure.

The mortality rate from porto-systemic shunts is approximately 10% for emergency cases and <5% in elective cases.

Prophylactic therapy

Currently there is no proven benefit from prophylactic sclerotherapy or band ligation but there are no randomised controlled trials in children. Prophylaxis with propranolol (1-5mg/kg/day) has been recently reported to be effective.[16]

Long term management

Long term management depends on the aetiology of portal hypertension. For children with extra hepatic portal hypertension with normal liver function the therapy is dictated by the frequency and severity of variceal bleeding. Ablation of varices with sclerotherapy or band ligation may be sufficient but porto-systemic shunt stunting now using the meso-portal shunt may be required for recurrent bleeding, severe growth failure or ascites.

In children with portal hypertension secondary to cirrhosis, the management depends on the stage of the liver disease. In children with compensated cirrhosis and no immediate requirement for liver transplantation, variceal ablation or insertion of a TIPSS may be adequate for short term measures but the development of significant hepatic dysfunction is an indication for liver transplantation.

Summary

Modern medical and surgical management of portal hypertension has improved outcome, but randomised trials are required to evaluate long term prognosis and therapy.

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Figure 1

Management of Acute Variceal Bleed in Childhood

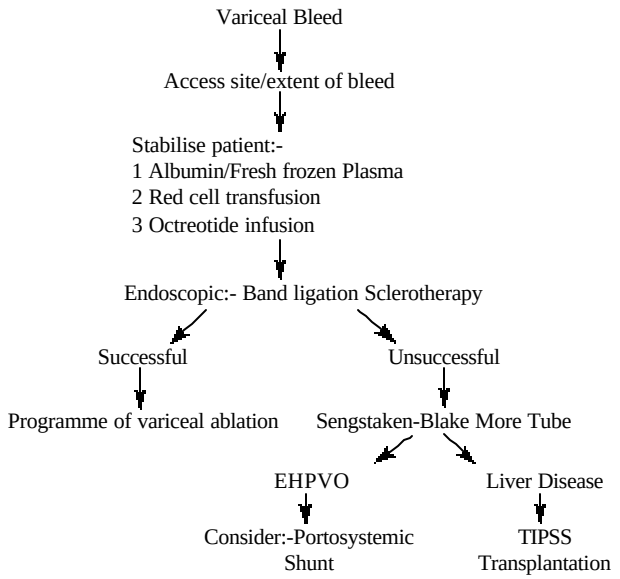

Table 2

Results of Porto Systemic Shunt Surgery in Children

	N	Patency	Follow Up
Meso caval	53	98%	35y
Spleno renal	55	90%	1-10y
Mesoportal	35	90%	6m-8y

Improving Results of Intestinal transplantation

Samuel Kocoshis, MD

LEARNING OBJECTIVES

As a result of this session:

1. The attendee should know the clinical findings predicting a poor prognosis among infants with short bowel syndrome.

2. The attendee should understand the rationale for and details of preemptive therapy for lympho-proliferative disease among intestinal transplant recipients.

3. The attendee should recognize the value of frequent protocol endoscopy and biopsy of allograft small bowel.

4. The attendee should recognize the implications of exfoliative rejection for future graft function among small bowel recipients.

Improving Results of Intestinal Transplantation

Samuel A. Kocoshis, MD

Introduction

Isolated intestinal, liver-intestinal, and multi-visceral transplantation had been performed as early as the 1960s, but only a handful of patients were transplanted prior to 1990 because primitive immunosuppressive regimens condemned intestinal transplantation to failure. Rejection or aggressive post-transplant lymphoproliferative disease (PTLD) resulted in the death of most patients within days to months after transplant.[1] In contrast, by 1990, the development of tacrolimus-based immunosuppression resulted in much better outcomes. Even though patient and graft losses remain substantial, the procedure is now successful enough to encourage surgeons to continue performing transplants while searching for methods to further improve outcomes.

Data from the large intestinal transplant programs suggest that survival has progressively improved through the decade of the 1990s. For example, the long-term survival of patients undergoing transplantation during the first year of the program at the University of Nebraska was only 17% (one survivor out of 6 patients). Over the past 6 years long term survival in the Nebraska series is 60%.[2] Similarly, at the University of Pittsburgh, actuarial 4-year graft survival has improved from 34% between 1990 and 1994 to 58% between 1995 and 1999.[3] In addition, cytomegalovirus (CMV) infection, PTLD, and graft versus host disease (GVHD) have decreased in prevalence from 40% to 29%, 32% to 15%, and 15% to 2%, respectively.[3]

Beyond improved survival, quality of life following transplantation is acceptable for both adults and children.[4] Furthermore, the vast majority of long term survivors can come off TPN and take full enteral or oral nutrition.[5,6] Several factors including patient selection, surgical technique, graft monitoring, immunosuppression, and preemptive antiviral therapy have impacted significantly upon survival and quality of life. Each of these factors shall be discussed.

Patient selection

The disorders experienced by adults referred for small bowel transplant are quite different from those experienced by pediatric patients. Most adults referred for transplantation suffer from ischemic bowel disease, Crohn's disease, desmoid tumors, and trauma whereas children most commonly suffer from gastroschisis, midgut volvulus, and necrotizing enterocolitis.[7] In either population, the mortality on the waiting list is greater than 40%.[7] Furthermore, pretransplant status has an impact upon survival, with sickest patients least likely to survive. Prognostic indicators for survival without transplant in pediatric patients on the waiting list have been analyzed by the Pittsburgh group at this meeting.[8] Clearly, infants with cholestasis and surgical short gut syndrome have the worst prognosis and the one-year survival is only 20% if they do not receive a small bowel transplant.[8] This has led to efforts to transplant them as soon as possible while simultaneously working to minimize the deleterious effects of TPN and bacterial overgrowth upon hepatic function. Such infants often are given choleretic drugs, undergo intestinal decontamination, have their parenteral protein load decreased, receive trophic feedings, and have their TPN cycled through the day.[9] Evidence for the efficacy of any of these measures remains controversial, but there are data to support their use.

Surgical technique

During the early 1990s, colon was included in the allograft when the candidate's colon was missing or deemed too short to adequately resorb water. This practice, in retrospect, was associated with a much higher rate of sepsis than was transplantation of small bowel alone. Furthermore, patient and graft survival are negatively impacted by transplanting colon. Therefore, abandonment of colon transplantation has had a significant positive effect upon survival.[10]

Virtually every small intestinal transplant recipient experiences abnormal allograft motility because of bowel denervation.[11] None experience the normal "fed pattern" of motility because migrating myoelectric complexes persist throughout the postprandial period. In addition, bowel compliance seems to be impaired and giant contractions appear randomly throughout the day. A strategy which may improve motility is the preservation of mesenteric ganglia as part of the allograft.

The shortage of suitable donors for liver/small bowel transplant candidates is distressing. Of the 40% of patients who die waiting for transplant, most die of liver failure. Employment of reduced-size en bloc liver and small bowel allogrofts with preservation of the common bile duct has been used successfully in several major transplant centers.[12,13] Inclusion of the intact pancreas in the composite graft may prevent the complications of partial pancreatectomy which frequently require reexploration of the patient.[14] *Enbloc* transplantation with or without pancreatectomy will undoutedly shorten waiting time for the large cohort of patients awaiting combined liver-small bowel transplant.

Graft Monitoring

In the 1980s and early 1990s, endoscopic graft monitoring for rejection was conducted on an ad hoc basis. As a result, rejection episodes were often identified too late to salvage graft mucosa, resulting in almost certain graft loss.[15,16] Currently, major transplant centers now maintain patients on a twice or thrice weekly regimen of protocol endoscopy and biopsy.[17] This strategy has clearly reduced the incidence of

exfoliative rejection at the University of Pittsburgh.

Immunosuppression

The conventional induction regimen for small intestinal transplantation has included high dose corticosteroids, tacrolimus, and azathiaprine as triple therapy.[1] Early transplants at the University of Pittsburgh also employed prostaglandin E1 as both an immunosuppressant and cytoprotective agent.[1] Use of prostaglandin has been abandoned because the agent appears to offer no advantage beyond conventional immunosuppression. Rejection episodes have been treated with high dose steroid boluses and recycling of steroids or (with a steroid resistant rejection) intravenous CD3 lymphocyte monoclonal antibody (OKT3) for seven to 14 days.

Since the early 1990s, immunosuppression has been modified somewhat. Immunosuppressive agents such as mycophenolic acid, rapamycin, and dacluzimab have permitted transplant teams to employ less toxic but more effective "cocktails" than originally used.

Mycophenolic acid (MMF) specifically prevents cellular DNA synthesis by blocking inosine monophosphate dehydrogenase. Because T and B lymphocytes are the cell types which cannot utilize the "salvage pathway" to synthesize DNA, MMF specifically blocks their proliferative response to antigens while permitting other cell types to replicate.[18] Even though MMF frequently produces diarrhea and sometimes produces bone marrow suppression, azathiapine resistant rejection episodes may respond to MMF. Conversely, MMF-resistant rejection may respond to azathiaprine.

The calcineurin inhibitors, cyclosporine and tacrolimus, have a proven track record as immunosuppressants. However, both are nephrotoxic. They inhibit transcription of cytokine messenger RNA with lymphocyte activation. In contrast, the macrocyclic lactone fermentation product, rapamycin, blocks a serine-threonine regulatory kinase (called target of rapamycin [TOR]) governing signal transduction from the cytokines followed by T-cell proliferation.[19] The use of both rapamycin and tacrolimus thus has a synergistic immunosuppressive effect by blocking both transcription of cytokine and downstream signaling pathways. Studies in renal allograft recipients confirm that rapamycin enhances the immunosuppressive effects of calcineurin inhibitors.[19] Furthermore rapamycin spares the kidney and permits reduction of maintenance tacrolimus doses, thereby improving long-term renal function in allograft recipients. Therefore, it will probably soon enter maintenance immunosuppression protocols for intestinal transplant recipients.

The proinflammatory cytokine, interleukin 2 (IL2), is crucial in the allograft rejection process. Increases in IL2 production directly result in increases in IL2 receptor (IL2R) levels. Hence, investigators have developed two monoclonal antibodies (dacluzimab and basiliximab) targeting the alpha chain (CD25) of IL2R in an effort to reduce T-cell proliferation and prevent rejection.[20] Preliminary data from phase II clinical trials among intestinal transplant recipients suggest that dacluzimab reduces both the incidence and severity of early allograft rejection when used during the induction phase. It may function as a salvage agent for established rejection as well.

An immunomodulatory strategy which has been utilized successfully at the Universities of Pittsburgh and elsewhere is the augmentation of donor lymphocyte and stem cell populations by perioperative bone marrow infusion at the time of small bowel transplant.[21] This strategy is safe, and data from both the University of Miami and the University of Pittsburgh suggest that this is associated with a reduced incidence of acute or chronic rejection as well as improved patient outcome. Central to the principle of bone marrow infusion is the enhancement of microchimerism. Though microchimerism is perceived by some investigators as an effect of tolerance rather than the pathway toward tolerance,[22] animal data from the University of Pittsburgh strongly suggest that progressive enhancement of the donor-derived dendritic cell pool achieved through bone marrow infusion will induce the clonal deletion of the killer T-cells,[23] which is essential to produce immune tolerance.

Finally, for isolated intestinal transplant (as opposed to a liver/intestine transplant or a multivisceral transplant), a negative lymphocytoxic crossmatch between donor and recipient will reduce the prevalence of severe rejection by 3½ fold.[24] Paradoxically, the negative impact of a positive crossmatch is not seen when patients receive liver-small bowel transplants. The implication of this phenomenon is that a transplanted liver has protective effects upon transplanted bowel.

Preemptive therapy for PTLD

The high prevalence of lymphoproliferative disease (14 cases out of 30 patients) among the first 30 small intestinal recipients at the University of Pittsburgh led to routine monitoring of viral load in blood combined with preemptive antiviral therapy when high viral loads are discovered.[25] All patients undergoing small bowel transplant at the University of Pittsburgh receive EBV polymerase chain reaction (PCR) testing every 2 weeks for the first 3 post transplant months. Subsequently, the frequency is decreased to once in 2 or 3 months. An EBV load of 40 copies/105 peripheral blood lymphocytes (PBL) for EBV seronegative patients or 200 copies/105 PBL for seropositive patients is defined as acquisition or reactivation of EBV, respectively. When EBV PCR has attained the critical level, ganciclovir and CMV-immunoglobulin (CMV-IVIG) are started, and immunosuppression reduced if possible. Gancyclovir is continued daily and CMV-IVIG every 2 weeks until the EBV levels fall to below the cutoff on 2 successive measurements.

The overall prevalence of PTLD fell from 14 out of 30 transplanted patients before initiation of the protocol to 7 out of 27 after initiation of the protocol. Even more importantly, only 3 out of 23 patients who adhered properly to the protocol developed PTLD. The prevalence of PTLD thus appears to be affected by close monitoring of viral load and with preemptive therapy.[25]

Future trends in intestinal transplantation

While the data from the University of Pittsburgh regarding actuarial survival of candidates referred for transplantation are

valuable,[8] the population studied is biased by the fact that referring physicians had already concluded that transplantation offered the best hope for survival. The probability of survival of long term parenteral nutrition recipients who were never referred for transplantation must be sought elsewhere. Unfortunately, the transplant surgeon and gastroenterologist possess a still too nebulous crystal ball with which to accurately predict which patients should be listed early for transplantation. Further refinement of multivariate risk analysis for patients on long term TPN is certain to appear published in the future.

Living related donation is likely to be utilized in the future if satisfactory vascular anastomses can be performed.

Immunosuppression and immunomodulation will certainly be more sophisticated in the next decade. Up until the present, murine monoclonal antibodies to lymphocytes have had disadvantages. They require frequent dosing because of short half lives. This, in turn, results in an immune response to them which produces unwanted side effects and limits efficacy. The newer, currently available murine monoclonal antibodies are now being humanized. Through genetically engineered fusing of the murine variable region to human immunoglobin constant region,[26] antibody binding remains unfettered while the half life is lengthened and the immunogenicity reduced to humans. Humanized CD3 antibodies will soon be available to replace the highly immunogenic OKT3.

At least four more novel immunosuppressive strategies are either in human trials or will soon be in trial.[27] Rather than inhibit CD4 activation, T-cell receptor binding of mixed histocompatability class 2 with monoclonal CD4 antibodies can be interrupted. Alternatively, T-cell costimulation which must take place for optimal IL2 production, can be blocked with anti-CD154 antibodies or CD28-B7 blockade. Adhesion molecules, critical for lymphocyte recruitment and activation, can also be blocked with monoclonal antibodies or with antisence molecules. Accessory molecules, such as lymphocyte function antigen 3 (LFA 3) or CD2 may be targeted by antibodies, and the protein tyrosine phosphatase (CD45) which is chemically inhibited by rapamycin can also be blocked by a monoclonal antibody.

Undoubtedly, enhancement of chimerism will also be perfected in the coming decade.

The best antiviral strategy is the development of a “cocktail” which engenders graft tolerance while minimizing immunosuppression. Until such a cocktail is developed, life threatening viral infections will continue. Therefore, antiviral therapy will continue to be necessary. Among the newer antiviral therapies, one of the most promising on the horizon is retuximab, an antibody against CD20, the surface antigen expressed by most mature and proliferating B lymphocytes.[28] This antibody spares plasma cells and stem cells so that immunoglobulin production continues; furthermore, B cells return to the circulation after cessation of therapy. Now employed for B cell lymphomas in adults, it shows promise for treating polyclonal lymphoproliferative disease in both children and adults.

Another anti-EBV strategy which has already attained limited use in solid organ recipients with established lymphoproliferative disease is the infusion of lymphokine activated cytotoxic T-cells harvested before transplant from either donors or EBV positive recipients.[29] Unfortunately, this approach has had greater usefulness in bone marrow transplantation than in solid organ transplantation because of the need to infuse histocompatible lymphocytes in solid organ recipients. Recent refinements in the understanding of dendritic cells makes their infusion a realistic possibility for EBV naïve patients. Efforts are also underway to perfect ex vivo clonal expansion of EBV positive cytotoxic lymphocytes in EBV positive organ recipients whose infection has reactivated.

Finally, research in Epstein-Barr and cytomegalovirus vaccines is rapidly progressing.[30,31] Epitope based vaccines are unlikely to be developed in the near future because of histocompatibility type polymorphisms and heterogeneous epitopes within viral strains. Structural glycoprotein-based CMV and EBV vaccines hybridized with a live-attenuated vaccinia carrier vaccine are likely to be the most successful of these.

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Notes

Use of Complementary and alternative medicine (CAM) in Pediatric Gastroenterology and Hepatology

Doris Strader, MD

LEARNING OBJECTIVES

At the completion of this session, participants will be able to:

1. Describe the use of Alternative Medications in the US over the past 3 years;

2. Compare and contrast Eastern vs Western medical philosophy as a means of understanding the increased use of Alternative medications in the US;

3. Describe the use of Alternative medications in the treatment of pediatric illness with a particular emphasis on biofeedback, homeopathy and herbal therapies.

Use of Complementary and Alternative Medicine (CAM) in Pediatric Gastroenterology and Hepatology

Doris B. Strader, MD

Introduction

For centuries, the use of herbal and botanical agents has been the cornerstone of medical treatment in many parts to the world. Recently, there has been increasing interest in traditional or "complementary and alternative" medical (CAM) therapies in the Western world, particularly in the treatment of diseases or conditions for which conventional, Western therapy is unsatisfactory. The term "complementary and alternative" is generally used to refer to those medical treatments not widely taught in Western medical schools or widely available in Western hospitals. CAM encompasses a variety of treatment modalities including herbal medications, folk remedies, megavitamins, homeopathy, hypnosis and imagery and prayer and spiritual healing. Many patients begin CAM treatments on the basis of recommendations from family or friends, or information obtained from magazine articles or the Internet, without the encouragement or knowledge of their physicians.

A recent study entitled "Trends in Alternative Medicine Use in the United States, 1990-1997" reported that the use of at least one alternative therapy by persons in the US has increased from 33.8% in 1990 to 42.1% in 1997.[1] In addition, the total out-of-pocket expenditures for CAM therapies in the US in 1997 was estimated to be $27 billion. These expenditures are comparable to the projected out-of-pocket expenditures for all US physician services during that same year. The largest increase in expenditures for CAM therapies has been for botanicals, for which the estimated cost in 1997 was $5.4 billion, a four-fold increase since 1990. These findings indicate that a large proportion of the US population is using CAM therapies and that this use is rivaling that of conventional medical treatments.

Paramount to understanding the use of CAM is the recognition that practitioners of "alternative" or traditional medicine have a different concept of how the human body functions and responds to its environment than do practitioners of Western medicine. In Western medicine the body is divided into compartments and function is measured by evaluating tissues and examining body fluids. Although there is a great deal of knowledge regarding the body's complex interactions, abnormalities are often diagnosed and treated as individual entities. Western physicians frequently sub-specialize focusing primarily on a particular compartment, and view disease as an invasion of the body by foreign organisms or a proliferation of individual cells out of proportion to their surrounding environment. The focus of Western medicine is to provide a cure for specific ailments. Toward that end, the scientific method is applied rigorously and claims of efficacy must be documented and proved by repeated independent study. Therapies and products are standardized and patients are treated according to "standard of care."

Traditional medicine, by contrast, teaches that energy flows through the body along energy paths, or meridians, and that this energy flows within, around and through all things in the universe.[2] Energy cannot be destroyed, but it can be negatively affected, leading to flow "imbalance" or disease. Traditional medicine does not view disease as an invasion of the body by foreign organisms, but rather, sees disease as "the human body out of balance." Healing, therefore, is the art of manipulating the flow of energy to re-establish balance in the whole person, rather than focusing solely on the area of complaint.

Spirituality is an integral part of traditional medicine and, as a result, traditional medical therapy can be very individualized, with no two persons receiving the exact same treatment despite very similar complaints. Herbs, when used properly, are considered benign yet essential for restoring the flow of energy in the acutely ill. Traditional medicine considers itself a preventive discipline and efficacy has been proven by centuries of trial and error and passed down by word of mouth.

An unpublished survey of CAM use among adult patients in six hepatology clinics across the US revealed a 42% prevalence of CAM use for all medical indications, with 23% of patients attempting to cure their liver disease with CAM.[3] The majority of patients continued to use the medications prescribed by their doctors in addition to CAM, claimed they mentioned the CAM use to their doctors and were neither encouraged or discouraged from using it, and used herbs, primarily milk thistle, to treat their liver disease. Data on the prevalence of CAM use to treat children with gastrointestinal and hepatic diseases is sketchy. It is clear that biofeedback has been an acceptable "alternative" therapy for the treatment of childhood functional and outlet obstruction constipation, fecal incontinence, and anorectal malformations for many years.[4-8]The prevalence of the use of herbs, homeopathy or acupuncture in treating children is unknown.

Biofeedback

The use of biofeedback in the treatment of constipation and fecal incontinence has been extensively studied. Results of these studies, however, have been equivocal. Briefly, biofeedback training is accomplished by having the patient lie on his/her right side facing an EMG display unit. A balloon is inserted into the rectum and inflated with 50mL of air so that the patients has the sensation of a full rectum and thus needs to defecate. Two electrodes are placed at the anal opening to assess external anal sphincter function. The patient is asked to strain as if having a bowel movement in an attempt to expel the balloon. The patient watches the monitor and notes the increase

in the trace amplitude from baseline indicating and increase in sphincter pressure. The patient is then asked to strain again without increasing sphincter activity so that the appearance of the amplitude of the tracing continues to resemble the baseline. (Patients with incontinence are taught the opposite; ie to voluntarily squeeze the sphincter with the goal of increasing pressure, as well as, the trace amplitude as long as possible). Certain requirements must exist in children for biofeedback to be effective.[9] First, there must be a well-defined response and easily measured response such as contraction or relaxation of the sphincter. Second, the end organ must be capable of responding, i.e., innervated. Third, there must be a perceptible cue for the patient to initiate control. And finally, there must be adequate motivation. This usually requires that the patient be at least 4 years old in order to cooperate with the training.

A 1996 review article evaluating eight studies of biofeedback in children with constipation concluded that biofeedback provided no benefits after the patient failed conventional treatment.[9] More recent studies involving children and adults, aged 11-96 years, show conflicting results. One study of 100 consecutive patients with fecal incontinence showed that 43 patients regarded themselves as symptomatically cured, 24 regarded themselves as symptomatically improved and 33 noted no change.[5] Anal ultrasonographic findings in 87 of the 100 patients revealed 37 patients cured (able to control sphincter activity), 29 improved and 21 with no change. A second study of 100 consecutive patients with constipation followed for a median of 23 months showed subjective improvement in 57%.[10] Patients with baseline slow and normal transit, males and females, those with and without paradoxical contraction of the anal sphincter on straining benefited equally from biofeedback. By contrast, a third study of 30 females with constipation revealed improvement in 9 patients, no change in 19 patients and 2 patients unable to complete therapy.[11]

In response to these less than optimistic reports, a number of investigators have attempted to identify factors that would predict a favorable response to biofeedback in patients with constipation. In a study of 194 patients, complete success, defined a passage of three or more spontaneous bowel movements per week with discontinuation of cathartics, was achieved in 35%.[12] Factors predictive of success were greater than five biofeedback sessions and the patients' willingness to complete the study. The above mentioned investigation of 30 females found equally poor complete response rates but suggested that success was more likely in patients without severe pelvic floor damage.[11] Neither of these studies included many children (none under age 11) and therefore caution should be used when trying to interpret these data in the pediatric population. Biofeedback is a relatively non-invasive treatment which may offer relief in well-chosen, motivated children with constipation or fecal incontinence and is being used in a number of institutions at the discretion of the physician. It is unclear whether further study will further elucidate the effectiveness of this treatment modality.

Homeopathy

Homeopathy is a discipline based on the premise that a substance which causes certain symptoms in healthy persons when given in large doses, can cure those same symptoms in ill persons when given in minute doses.[13] An examination which includes determining the emotional state of the patient, the color and odor of the stool, the presence or absence of abdominal pain, nausea or vomiting, the body temperature and degree of thirst, is performed. A combination of homeopathic medications is then chosen. The homeopathic medicines are made from plant, animal and mineral sources and are diluted in water/alcohol solution to extremely small concentrations. This has led many physicians to reject the homeopathic theory by stating that medications in infinitesimal concentrations cannot maintain therapeutic effect. Recently, however, clinical trials have suggested that homeopathy may be beneficial in the treatment of several illnesses when compared with placebo.

A well-known, randomized study of homeopathy vs. placebo in the treatment of childhood diarrhea in Nicaragua was published in 1994.[14] Eighty-one children with acute diarrhea, aged 6 months to 5 years, were randomized to receive individualized homeopathic medications plus oral rehydration or placebo plus oral rehydration. The authors reported a significant decrease ($P=0.048$) in the duration of diarrhea (i.e., number of days until there were less than three unformed stools per day for two days) and in the number of stools per day after 72 hours ($P=0.036$) in the treated group. It was concluded that homeopathy might be of value in the treatment of childhood diarrhea.

A number of criticisms of the study were quickly advanced.[15] It was suggested that (1) the method used to diagnose the type of diarrhea and decide on the combination of homeopathic medications was unproven, (2) the treatment was too individualized with patients receiving an average of 8 medications, and (3) the differences found were barely statistically significant. The first two criticisms are unwarranted in that the methods used in the diagnostic and decision-making tree of homeopathy must be given some regard, even if they are unproven in Western medicine. The third criticism attempts to suggest that had the population been larger, the differences would not have been significant. Although this may be true, the statistics were properly obtained, significantly different and thereby valid. At present, no larger trials of homeopathy in childhood diarrhea have been performed.

Herbal Treatments

To my knowledge there are no published data in Western literature regarding the use of CAM in the treatment of gastrointestinal or hepatic diseases in children. By contrast, a number of articles have been published in Asian and Eastern European journals detailing the effective use of herbal preparations for non-specific ulcerative colitis, rotaviral enteritis, chronic diarrhea, hepatitis B and peptic ulcer disease.

In one study from Jiangsu Province, China, 162 children with chronic protracted diarrhea (15-60 days prior to entering study) were randomly divided into two groups.[16] The ages ranged from 46 days to 3 years, and the causes of the diarrhea were improper feeding, acute or chronic enteritis and "exposure to

cold." One group received the Chinese herbal preparation Xiang Cheng San (a preparation containing several herbs including fructus listeae, flos caryophylli and cortex cinnamomi), applied externally to the umbilicus every other day for one week, while the second group received routine Western medications (antidiarrheals, antibiotics and gastric acid pills). Sixty-one of the 115 children in the herbal treatment group (53%) were cured, 31/115 (27%) improved and 23/115 (20%) had no effect. Among the 47 children treated with Western medications, 14 (30%) were cured, 17/47 (36.2%) were improved and 16/47 (34%) had no effect ($P<0.05$). Animal studies showed that external application of Xiang Cheng San may inhibit hyperperistalsis of intestines in mice and dilate microvessels in rats, thus improving the microcirculation of the mesentery. No toxic effects of the herbal preparation were reported.

In a study of peptic ulcer disease, 80 children with endoscopic or radiographic evidence of gastric or duodenal ulcers were treated with a Chinese recipe containing the herbs herba agastachis, fructus foeniculi, massa fermentata medicinalis, fu longgan, indigo naturalis and radix arnebiae.[17] After 8 weeks of treatment 65/80 (81.2%) ulcers were cured (i.e., clinical signs disappeared, occult tests were negative x 3 and healing was seen via endoscopy). Nine of 80 patients showed clinical improvement without healing of ulcers and in six cases there was no improvement or aggravation of symptoms.

Finally, a study of a Japanese Kampo formula, Sho-saiko-to (containing bupleurum, licorice root, ginseng root, skullcap root, jujube fruit, ginger root and half summer root) was administered to 14 children, ages 1 to 14.5 years, with chronic hepatitis B.[18] Seven of the 14 children (50%) became HBeAg negative during the average observation period of 6 months (range 0.2 to 0.9 years), and four of the seven responders developed anti-HBe. This 50% HBeAg clearance rate was higher than the 22.7% natural annual clearance rate seen in 22 untreated controls. In addition, 6 children treated with the herbal formula had normalization of ALT during the 12 month treatment period. The authors concluded that Sho-saiko-to shortens the conversion rate of HBeAg-positive hepatitis B to anti-HBe hepatitis.

All three of the above mentioned studies suffered criticism because the diseases evaluated are self-limited with a relatively short natural history. It is unclear whether the addition of herbal medications hastened the resolution of the illnesses or whether the herbs were merely bystanders in diseases which were already on the road to recovery. Although two of the studies used control subjects, the numbers of patients were small. Further study using larger numbers of patients is warranted.

Conclusion

The prevalence of CAM use in children with gastrointestinal diseases in the US is unknown. A number of European and Asian studies describe the benefit of biofeedback, homeopathy and herbal treatments in a number of diseases including diarrhea, incontinence, peptic ulcer disease and hepatitis. Unfortunately, many of the studies suffer from a lack of randomization, small numbers of patients and definitions of response which are subjective. Randomized, controlled trials with adequate numbers of patients and objective measures of response would help answer the question of the benefit of CAM in the pediatric population.

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Notes