Chronic Myelocytic Leukemia – Part II: Approaches to and Molecular Monitoring of Therapy

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DATA SOURCES: Current literature.

DATA SYNTHESIS: Chronic myelocytic leukemia (CML) was initially described in 1845 and is considered one of the first leukemias discovered. Effective approaches to therapy were not instituted until arsenic was first administered in 1865. Since then, four major therapeutic milestones have been achieved; the development of alkylating agents like busulphan and 6-thioguanine in 1953, alpha interferon in 1983, bone marrow transplantation in 1986, and tyrosine kinase inhibitors in 1998. The discovery that the protein product of this fusion gene expresses constitutive tyrosine kinase activity prompted the synthesis of a designer drug, imatinib mesylate, which binds the fusion protein and neutralizes the tyrosine kinase activity. Molecular methods of detecting BCR-ABL transcripts are showing promise in confirming drug resistance and predicting patient outcomes in response to imatinib mesylate therapy. Evidence of drug resistance can guide physicians in selecting alternative therapeutic approaches early in the course of the disease to potentially rescue non responders. Although the success of clinical trials has been dramatic, drug resistance and disease relapse are issues to be considered.

CONCLUSION: The discovery that the BCR/ABL fusion protein exhibits increased and constitutive tyrosine kinase activity led investigators to develop an inhibitor to this activity. The synthesis of imatinib mesylate, currently marketed as Gleevec™ or Glivec®, is in stage III clinical trials and has proven to be the most successful antileukemic drug to date. As in CML, an understanding of the leukemogenic mechanisms involved in other leukemias will provide the groundwork for the development of therapeutic interventions tailored to the specific molecular defects identified, eventually rendering obsolete the shotgun approaches to massive cell killing produced by chemotherapy.

ABBREVIATIONS: ABL = Ableson oncogene found in a strain of mouse leukemia virus; ASH = American Society of Hematology Annual Meeting; BCR = breakpoint cluster region; CCR = complete cytogenetic response; CHR = complete hematologic response; CML = chronic myelocytic leukemia; INF = interferon; MCR = major cytogenetic response; MMR = major molecular response; RT-PCR = reverse transcriptase-polymerase chain reaction; SCT = stem cell transplant.

INDEX TERMS: BCR/ABL; chronic myelocytic leukemia; Philadelphia chromosome; t(9;22); tyrosine kinase inhibitor.

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Focus Continuing Education Credit*: see pages 57 to 69 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES: Following careful study of this review, the reader will be able to:

1. Describe the first documented therapy for CML.
2. Discuss the chemotherapeutic approach to CML treatment.
3. Discuss one advantage and one disadvantage of alpha interferon and bone marrow/stem cell transplants in the treatment of CML.
4. Discuss the therapeutic approach to CML involving tyrosine kinase inhibitors to include:
   a. molecular target of tyrosine kinase inhibitors.
   b. function of the tyrosine kinase inhibitors.
   c. effectiveness of therapy.
   d. drug resistance and adverse events.
   e. alternative therapeutic approaches in patients with drug resistance.

EVOLUTION OF THERAPEUTIC APPROACHES TO CML

The approach to CML therapy has evolved over the years and is on the threshold of a potential cure for many patients. The first documented therapy that was effective for CML was arsenic, first administered in 1865 by a German physician named Lissauer.[1] In this patient, arsenic therapy was shown to reduce the WBC count, diminish the splenomegaly, and improve anemia, all contributing to the amelioration of symptoms. This therapeutic approach was continued until 1903 when radiotherapy was instituted.[2] In 1912, benzene was introduced in conjunction with radiotherapy as a treatment for CML.[3] An alternative form of radiotherapy using radioactive phosphorous was initiated in 1938.[4]

Nitrogen mustards, an agent of chemical warfare, were administered to CML patients in 1947.[5,6] These experiments with nitrogen mustards led to the development of other alkylating agents, such as busulfan which was introduced in 1953, and ushered in the modern era of chemotherapy.[7] Long-term survival was improved for the first time and busulfan combined with 6-thioguanine became the mainstay of treatment for the next 35 years. Alpha interferon was introduced in 1983 as a therapeutic approach that not only increased survival, but it also induced Philadelphia chromosome negativity and reduced progression to blast crisis.[8] This therapy was supplemented with bone marrow transplantation in 1986 for patients under 55 years of age and became the mainstay until recently.[9] The future direction of therapy lies in tyrosine kinase inhibitors and other molecules that block key steps in the leukemogenic signal transduction pathways leading to full transformation.

CML THERAPY IN THE MODERN ERA

Until recently, the standard approach to CML therapy was to reduce the tumor burden with tumor reducing chemotherapy. Although CML precursors were engaged in cell division to a greater extent than normal myeloid stem cells, they were not active enough to be significantly affected by aggressive chemotherapy, as is used in acute leukemias. The more commonly used drugs to treat CML were busulfan, hydroxyurea, 6-mercaptopurine, and 6-thioguanine. Hematologic remissions were achieved in 75% of patients treated with busulfan or hydroxyurea but these remissions only lasted two to three years. Although hydroxyurea is preferred over busulfan due to lesser toxicities and prolonged survivals, (56 months vs. 44 months respectively), neither drug produced a clinically acceptable rate of cytogenetic response or slowed disease progression toward blast crisis.[10, 11]

The advent of alpha interferon (INF-α) improved outcomes in CML patients when used as initial therapy by inducing cytogenetic remissions and increasing survival rates from 35% to 55%.[12] These findings earned INF-α the position of first-line therapy in CML patients who were not eligible for allogeneic bone marrow transplantation. INF-α reduces myeloid cell numbers by stimulating a cell-mediated, anti-tumor host immune response. INF-α also induces apoptosis in the leukemic clone and restores integrin-mediated adhesion to collagen. Binding of CML progenitors to bone marrow stroma induces natural proliferation inhibition mechanisms.[13] INF-α has improved the rate and duration of hematologic remissions to between 60% to 80%, produced major cytogenetic responses (MCR) (<35% Ph1+ metaphases detected) in 25% of cases and produced complete cytogenetic remission (CCR), defined as Ph1 negativity, in 10% to 20% of the cases.

Dramatically improved response rates can be achieved when cytarabine (Ara-C) is administered with INF-α.[14] At 18 months post therapy, the INF-α with Ara-C arm of the IRIS (International Randomized INF vs. STI571) study revealed a hematological remission rate of 93%, an MCR of 34%, and a CCR of 15%. However, some patients experienced severe side effects, developed drug resistance, and succumbed to relapses at rates similar to chemotherapy. Most important, the majority of patients achieving CCR following interferon therapy retained the BCR-ABL gene and its products as detected by molecular methods like RT-PCR and fluorescent in situ hybridization (FISH).[15]

As techniques improved, bone marrow and stem cell transplants have become a viable form of therapy, especially in younger patients in the chronic phase of CML, due to the curative potential of the treatment. Relapses occur in 15% to 30% of transplanted patients but relapses become infrequent beyond the five-year survival threshold. Long-term survivals were reported between 50% and 80% with disease free survival rates between 30% and 70%. Normal hematopoietic progenitors exist in the CD34(+), HLA-DR(-) cell pool and can be mobilized from autologous and allogeneic donors for stem cell transplants. To be considered a good candidate for transplantation, patients need to be less than 50 years old, in the chronic phase of the disease, and within one year of diagnosis. The patient would receive ablative chemoradiotherapy, followed by transplant. Unfortunately, the combination of limited numbers of HLA compatible related donors and age limitations of CML patients, results in only 15% to 20% of CML patients qualifying for transplantation. If HLA matched unrelated donors are considered, the candidacy rate for transplantation increases to 30%.[16]

In contrast, autologous transplants usually result in relapse within one year, due to residual disease in the patient, or in the bone marrow preparation. If bone marrow transplant is performed in the chronic phase of the disease, the five-year survival rate is 50%. This statistic drops to 30% if performed in the accelerated phase, and to 15%, if performed in blast crisis phase. Stem cell transplant remains the treatment of choice for young patients (<40years) with CML who have HLA-identical siblings. The three-year survival rates for unrelated donors matched for HLA-A, HLA-B, and HLA-DR are improving for both young patients (68%) and patients between the ages of 40 and 55 (67%). Progress has been made in the success of autologous stem cell transplants by purging the stem cell product of malignant cells and by enriching for normal stem cells that are CD34(+) and HLA-DR(-). Donor lymphocyte infusions (DLI) have produced durable complete remissions in >70% of patients who have relapsed following allogeneic transplantation with a sibling donor. The mechanism of activity in DLI appears to be T cells directed at CD34(+) CML progenitor cells.[16]

The future of CML therapy appears to be focusing on specific molecular targets that block signal transduction pathways altered by the BCR-ABL fusion protein. The first and most promising therapeutic intervention involves synthetic tyrosine kinase inhibitors. Since most, if not all, of the transforming capability of the fusion protein stems from its tyrosine kinase activity, selective inhibition of this activity has proven successful. STI-571, formerly called CGP57148, is a synthetic tyrosine kinase inhibitor designed to selectively inhibit the tyrosine kinase activity of the BCR/ABL fusion protein by binding the ATP binding cleft. Binding of STI-571 to the ATP binding cleft blocks the binding of ATP thus preventing the abnormal phosphorylation events caused by the BCR-ABL fusion protein.

Phase I/II clinical trials involving CML patients resistant to interferon have been underway since June 1998 and the results look very promising. When given as a 300+ mg oral daily dose, all CML patients achieved complete hematological remissions (defined as a normal white blood cell count) with some cytogenetic remissions in three weeks of therapy. In many cases, even patients who failed standard INF-α therapy have achieved complete hematologic remission. The drug must be taken daily because it has a short half-life of 12-14 hours. Some patients achieved cytogenetic responses suggesting that the therapy may be inducing apoptosis in the malignant clone. STI-571 would be considered effective if its only function was to inhibit the tyrosine kinase activity that causes the malignant phenotype, but if it also kills the malignant cells by inducing apoptosis, the effectiveness is dramatically amplified. The drug appears to have minimal side effects and no dose-limiting toxicities have been encountered.[17] STI-571 is also called imatinib mesylate (imatinib) and is manufactured commercially by Novartis Pharmaceutical under the name Glivec^{R} or Gleevec^{TM}. Studies are underway to maximize imatinib dosing, to combine imatinib with other therapies, and to monitor the effectiveness of therapy using molecular monitoring techniques.

MOLECULAR MONITORING OF THERAPEUTIC RESPONSES

The most effective molecular monitoring approach to date involves the quantitation of BCR-ABL transcripts that remain in blood after therapy. Competitive or real-time quantitative reverse transcriptase PCR (Q-PCR) was first used to monitor patients receiving INF-α+ or allogeneic stem cell transplants (allo-SCT).[18-21] Results demonstrated a correlation between the copy number of BCR-ABL transcripts in the blood and the Ph1+ metaphases in the bone marrow for patients on INF-α and was a predictor of cytogenetic and hematologic relapse in patients who received allo-SCT.[21-23] As with INF-α, Q-PCR has also demonstrated close correlation between Ph1+ metaphases in bone marrow and BCR-ABL transcripts in peripheral blood from patients receiving imatinib therapy.[24,25] One study reported that 28 patients who achieved CCR following imatinib therapy had <1% BCR-ABL transcripts with only one exception, whereas of the 48 patients not achieving CCR only two had <1% BCR-ABL transcripts in the peripheral blood.[26] This work was corroborated by a second study that reported that 40 of 42 patients who achieved CCR demonstrated BCR-ABL transcripts in blood of <2%.[27]

Laboratories are beginning to report BCR-ABL copy number from peripheral blood in terms of the number of log reductions in copy number compared to the patient's original, pre-treatment copy number (baseline). This is a more universal reporting method that partially corrects for differences in testing sensitivities between labs and in initial tumor burden between CML patients. Using this nomenclature, the IRIS study defined a major cytogenetic response as a 3-log reduction or greater in BCR-ABL transcripts detectable in peripheral blood. Maximum sensitivity for most assays is at least 4.5-logs below baseline so maximum measurable response was defined by the IRIS study as 4.5-logs below baseline. Although molecular monitoring of BCR-ABL appears to correlate with karyotyping analysis and is predic-

tive of therapeutic response, karyotype analysis is still valuable. Chromosomal abnormalities, other than Ph1, occur in some CML patients treated with imatinib as first-line therapy and are predictors of disease progression, thus warranting periodic karyotype analysis.[28]

EFFECTIVENESS OF IMATINIB THERAPY AS MEASURED BY MOLECULAR METHODS

Although hematological remissions are extremely high when imatinib is given as first-line therapy, major molecular responses (MMR), as defined as a >3-log reduction in BCR-ABL transcripts from baseline, are more difficult to achieve. The IRIS study group reported that 39% of newly diagnosed CML patients achieved MMR after 12 months of imatinib therapy. This is a more impressive result when compared to a MMR of 2% in the patients treated with a combination of INF-α and Ara-C. In addition, 20% of patients demonstrated a 2- to 3-log reduction in BCR-ABL transcripts from baseline and 19% achieved a 4-log or greater reduction following 12 months of imatinib therapy. Maximum molecular response of >4.5-log reduction was also observed in other smaller studies.[27] It is expected that the MMR is likely to increase as patients on first-line imatinib therapy are followed beyond one year. Data suggest that CCR and MMR are good predictors of disease progression. Among all the patients in the IRIS study that achieved CCR on imatinib, 58% also achieved MMR and none of these patients showed disease progression in the subsequent 12-month follow-up period. In addition, there is only a 15% probability of disease progression among the imatinib-treated patients who did not achieve CCR, and a 3% among those who achieved CCR but not MMR.

The primary goal of imatinib therapy is to achieve undetectable levels of BCR-ABL transcripts in the blood that would produce ongoing remission and avoid disease progression. However, it is unclear if imatinib therapy alone can permanently disable the BCR-ABL leukemogenic pathway and/or eliminate BCR-ABL bearing CML cells through apoptosis or other mechanisms. Eventually, imatinib may need to be withdrawn from patients who have achieved long-term MMR (at undetectable BCR-ABL levels) and followed to determine if reemergence of BCR-ABL transcripts will occur. As for the present, it is of much greater concern that strategies be developed to address patients who have lost remission through the generation of additional cytogenetic abnormalities or resistance to imatinib therapy.

IMATINIB RESISTANCE AND DOSING

Primary resistance to imatinib has been defined as newly diagnosed CML patients who do not achieve complete hematologic remission (CHR) by three months, MCR by six months, or CCR by twelve months. In the IRIS study using newly diagnosed CML patients on 400 mg/day imatimib, 4% did not achieve CHR at three months, 23% did not reach MCR at six months and 31% failed to attain CCR at twelve months. These imatinib resistant patients represented between 20% to 30% of the study group.[29] However, in patients treated with 800 mg/day imatinib who had previously failed on INF-α, all 36 achieved CHR and only 11% did not reach CCR.[30] Some experts predict that approximately 90% of newly diagnosed CML patients might achieve CCR given higher doses of imatinib. Nevertheless, the 10% of patients predicted of having primary resistance can't be explained by the currently identified mutations and polymorphisms in BCR-ABL.[31]

Acquired resistance to imatinib is defined as a loss of a previously established response (CHR, MCR, CCR) or progression of disease and is a much greater problem. In the IRIS study, 8% of patients treated with imatinib developed resistance in 18 months while other studies report patients who began imatinib therapy both early and late in the chronic phase of CML who developed resistance at a rate of 15% and 25%, respectively, after 14 months.[29,31] There are two dominant theories that explain acquired resistance to imatinib; expansion of CML cells with transforming mechanisms independent of the BCR-ABL protein or genetic lesions that have altered the BCR-ABL protein either quantitatively or qualitatively. An example of the former theory would involve CML cells that previously had or have since developed additional genetic mutations, whether detectable or not, that possess transforming capabilities independent of BCR-ABL. However, the latter explanation is the more common scenario. Over 23 distinct point mutations have been identified in the BCR-ABL kinase domain and associated with imatinib resistance. The incidence of point mutations increases with the duration of the disease.

This suggests that the CML clone is incurring sequence errors during DNA replication, which is consistent with most cancer models. These point mutations are presumably occurring in multiple loci within the genome but many have been identified in the BCR-ABL kinase domain. The mutations are not necessarily stimulated by imatinib because similar mutations have been identified in many patients who have had CML for months to years but have never been exposed to imatinib.

However, these mutations are affecting the binding affinity of imatinib, resulting in resistance. The mutant clones will either not bind imatinib or bind it at a lower affinity resulting in continued proliferation of CML cells in the presence of imatinib. This process will select for imatinib resistance clones and promote disease progression.[31-33] It is unclear if the control of cell proliferation initially induced by imatinib when administered early in the course of the disease will reduce mutations that produce imatinib resistance.

A potential strategy to rescue CML patients who have developed imatinib resistance involves the identification of the particular BCR-ABL mutation present and the administration of specific tyrosine kinase inhibitors unaffected or less affected by that mutation. For those mutations that abrogate imatinib binding, imatinib should be discontinued or used in combination with another tyrosine kinase inhibitor less affected by the mutation. In cases where the binding affinity of imatinib is diminished, dose escalation may prove successful. [31-33] Prognosis for imatinib resistant patients appears particularly poor if the mutation is in the P-loop of the ATP phosphate binding domain.[31] However, a class of small molecule kinase inhibitors, called pyrido-pyrimidines, are currently being tested to determine kinase inhibition in both wild-type BCR-ABL proteins and in BCR-ABL mutants that have developed acquired imatinib resistance. These molecules are potent tyrosine kinase inhibitors and appear to bind the ATP binding site of BCR-ABL using different contact points as compared to imatinib. Von Bubnoff tested 13 different pyrido-pyrimidines in a murine system and found all to exhibit tyrosine kinase inhibition and reverse the CML phenotype.[34] However, three of these molecules exhibited potent tyrosine kinase inhibition: SKI DV-2-43, PD166326, and SKI DV-M016.[34] In preliminary studies using a mouse model of CML, PD166326 inhibited BCR-ABL tyrosine kinase activity between 4 and 100 times greater than imatinib. Further studies are underway to determine the effectiveness of PD166326 at treating CML without crippling normal tyrosine kinase pathways and without inducing drug related sequalae.[35]

Three additional therapeutic approaches for patients who have developed imatinib resistance are to combine imatinib with drugs that inhibit other enzyme systems downstream of the tyrosine kinase activity of BCR-ABL, with chemotherapeutic drugs, or with CML vaccines. O'Hare found that a Src/Abl kinase inhibitor, AP23464, exhibited an 8-fold greater inhibition of BCR-ABL than imatinib using wild-type expressing Ba/F3 cells.[36] In addition, it also inhibited several other cell lines expressing common imatinib resistant BCR-ABL mutations.[36] Two farnesyl transferase inhibitors, Lonafarnib (SCH66336)[37] and Tibifarnib (Zarnestra™, R115777)[38] have been shown to reverse the CML phenotype in imatinib resistant patients when administered with imatinib. Favorable results have also been achieved in imatinib resistant patients when combining chemotherapeutic agents like semi-synthetic homoharringtonine (Myelostat®) and 5-aza-2'-deoxycytidine (decitabine) with imatinib.[39,40] A CML vaccine, CMLVAX100, has been shown to reduce the number of Ph1 positive cells in patients treated with imatinib who were exhibiting consistent residual disease. Over half of the patients achieved CCR and half of these CCR patients were negative for BCR-ABL transcripts by real-time quantitative PCR.[41]

STRATEGIES FOR THE MANAGEMENT OF CML PATIENTS

Even in light of the promising results achieved with imatinib, the most appropriate therapeutic approach to patients newly diagnosed with CML is still under discussion. It is clear that imatinib therapy has proven superior to the previously established treatment regimen using INF-α and Ara-C. However, several questions concerning imatinib therapy still remain. First, what are the long-term outcomes for patients who respond to imatinib therapy? Are they cured or will some, many, or all eventually succumb to some form of drug intolerance or resistance resulting in relapse? How should imatinib non-responders be treated? What criteria should be applied to imatinib treated patients to distinguish responders from non-responders to determine the efficacy of continuing imatinib therapy? In addition, allo-SCT has proven successful and potentially curative in a subset of CML patients. However, in contrast to the safety of imatinib, allo-SCT incurs the risk of transplant-related mortality, morbidity, and chronic graft-versus-host disease, limiting the procedure to only low risk candidates. What criteria should be used to determine which patients are better served by allo-SCT versus imatinib therapy?

It seems clear that there is a subset of patients who hold the hope of cure if they receive an uncomplicated allo-SCT. The ideal candidate for allo-SCT is a CML patient who is less than 40 years old, in the chronic phase of the disease, and within one year of diagnosis, and has an HLA-identical donor. The decision is more complicated if the patient is slightly older, just outside the one-year diagnosis window, or the donor is not a perfect HLA match. Other variables that complicate the decision to proceed with allo-SCT include recipient/donor gender combinations and CMV serostatus.[42,43] Once

the patient's clinical features exceed these parameters, imatinib therapy is likely to be the better choice. Allo-SCT may also be a viable option for CML patients who are expected to show imatinib resistance or in patients who have failed imatinib therapy. Currently, there are no reliable markers to predict imatinib resistance but mutations in the ATP binding site of the BCR/ABL fusion protein will produce such a resistance. Another version of transplantation that has shown success is the reduced intensity conditioning transplantation (RIST). The procedure is designed to gradually ablate host hematopoietic tissues by donor T cells and can be administered as an original graft, given again in the post-transplant period or by additional lymphocyte infusions. One study reported an overall disease free survival of 85% at five-year post RIST with all survivors being negative for BCR-ABL by RT-PCR.[44] Some argue that a strategy that combines the effects of imatinib and allo-SCT may be a viable approach. Since imatinib produces a more durable and complete remission than INF-α, it is possible that allo-SCT outcomes may be improved if imatinib is used as the pre-transplant conditioning regimen instead of INF-α. Another combination approach may be to use imatinib following allo-SCT to reduce residual disease. This approach has not been studied as a primary strategy but has been successful when performed as a rescue procedure following the failure of allo-SCT therapy.[45]

Therapeutic approaches to patients who failed to respond to front-line imatinib therapy or who have lost remission is also under debate. Data suggest that there are at least five possible approaches to this problem: 1) increasing the dose of imatinib to 600 or 800mg/day[46,47]; 2) adding another agent to the imatinib regimen like INF-α, Ara-C, hydroxyurea, decitabine, or homoharringtonine[48,49]; 3) changing the treatment to one or more of these agents without imatinib[50]; 4) autologous transplant[51]; and 5) new tyrosine kinase inhibitors. Adoptive immunotherapy (vaccines) approaches are currently being investigated to include using the e14a2 fusion protein, proteinase 3, Wilm's tumor protein, and heat shock protein 70 (Hsp-70) as the vaccine material but results are not as promising as hoped.[52-55]

In summary, it seems reasonable to start all newly diagnosed patients on imatinib and measure their hematologic, cytogenetic, and molecular response at 12 months. Those patients who are responding and meet the criteria for allo-SCT would be given that option. Those who are responding to imatinib but do not meet the allo-SCT criteria would continue on imatinib therapy. Those who demonstrate primary resistance to imatinib by not achieving hematologic remission at three months, MCR by six months, and CCR by twelve months would be dose escalated on imatinib, treated with a combination of drugs with or without imatinib, or potentially started on a next generation tyrosine kinase inhibitor. For any given patient with imatinib resistance, selection of the appropriate next generation tyrosine kinase inhibitor would be based on the identification of the mutation that produced the imatinib resistance. If remission is achieved through second-line therapy, patients would be evaluated as a transplant candidate. If patients are not eligible for transplant, second-line therapy would continue. This same strategy could be applied to patients who achieve remission but eventually develop an acquired resistance to imatinib. Autologous transplant could also be considered for those patients experiencing imatinib resistance.

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Book Review

Cases in Human Parasitology
by Judith S Heelan
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ISBN 1-55581-296-1
Paperback: 243 pp, $59.95

Not since Reifsnyder's *Parasitic Diseases Case Studies* (1980) has a book of parasitic disease case studies been published. A welcomed edition, Heelan's *Cases in Human Parasitology* includes cases of emerging, as well as classical parasites, along with wonderfully colored photomicrographs of the organisms in question. As stated in the introduction, the purpose of the book is to "present cases solely involving parasites to supplement conventional textbooks in human parasitology and to provide an interesting and educational challenge to health care scientists." The book contains 62 cases of patients who presented to an emergency department or to their physician with symptoms of a parasitic infection.

The book is divided into five sections: Intestinal Protozoa; Blood and Tissue Protozoa; Cestodes, Trematodes, and Intestinal Nematodes; Blood and Tissue Nematodes; and Challenging Cases. The latter section also includes some infections in patients with symptoms closely resembling parasitic infection. A glossary is also available at the end of the book. Each section is preceded by a concisely written introduction of background information and ends with a reference list. Each case includes a brief presentation of pertinent patient history appropriate to the infection—travel history, symptoms, age of patient, season, and characteristics of the organism in question, accompanied by a photomicrograph. The history is followed by a list of questions suggesting topics discussed in a comprehensive parasitic textbook; such as, identification, epidemiology, treatment, life cycle, transmission, prevention, and control. The question section is followed by concise answers.

This would be an ideal book for use in a human/medical parasitology course whether for clinical laboratory science students, medical students, infectious disease residents, clinical pathology residents, or even biology undergraduates. It could easily be adapted because its sectional organization is similar to that of most parasitology courses. Since many health curricula include case-based approach, Heelan's text would be an excellent tool for such. Individuals preparing for national examinations should also find *Cases in Human Parasitology* an excellent means for reviewing the topic. I highly recommend the book.

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