

Prognostic Factors

Making sense of prognostic factors in CLL

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Natural history of CLL

Like many other types of indolent non-Hodgkin's lymphoma, CLL is cancer of immune cells called B lymphocytes. However, the exact identity of the normal counterpart of the CLL cell is still unclear and we do not know what actually causes the disease. The current idea is that CLL probably starts as an immune response, perhaps to a bacteria or virus, which for some reason is not switched off and over time turns into a malignant process.

CLL is usually a slowly growing cancer. It almost always involves the blood and bone marrow and sometimes also the lymph nodes, liver and spleen. Problems can arise for several reasons. One of the most important complications of CLL is malfunctioning of the immune system. This can take two forms: under-activity against things it should be active against (e.g. bacteria, fungi and viruses) and over-activity against things it should not be active against (e.g. red cells and platelets). Another problem that can occur is that the bone marrow can become so crowded with CLL cells that its function becomes impaired. This results in the reduced production of red cells, platelets and normal white blood cells. Finally, the lymph nodes, liver or spleen can become enlarged to the point of becoming uncomfortable, and enlarged lymph nodes can press on important internal structures.

Treatment is usually reserved for patients with symptoms, rapid progression or complications, as there is no current evidence that treating patients without any of these features does any good. Chemotherapy is effective in most patients to begin with. However, relapse is inevitable. Many patients respond to treatment the second and third time round but the chances of a good response become lower each time the disease comes back, and eventually the disease becomes resistant to therapy. A significant proportion of patients with progressive CLL eventually succumb to infection due to impaired immunity.

Variability between patients

One of the striking things about CLL is that it behaves very differently in different patients. For example, some lucky patients have a very gentle form of the disease that never causes any symptoms or complications and never requires any treatment. At the other end of the spectrum, other less fortunate patients have an aggressive form of the disease that progresses rapidly and does not respond that well to chemotherapy. Many patients and their relatives find it useful to have some idea about how their disease is likely to behave as it gives them the opportunity to plan their lives accordingly. There are a number of ways of obtaining prognostic information ranging from very simple things to very complex tests that can only be done in a small number of specialist units. This article will review these various 'prognostic tests'.

Clinical staging

This is an old-fashioned but nevertheless very powerful way of predicting outcome in CLL. The idea here is that patients who already have a lot of disease on board when they are diagnosed must have a form of CLL that has grown relatively quickly and which is likely to continue growing quickly and behave in a generally aggressive way, and this turns out to be right. There are two staging systems in use. The Binet system (named after its French inventor) is the one most commonly used in Europe and the UK, while the Rai (named after its North American inventor) is



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the system most commonly used in the USA. Essentially, Binet Stage C or Rai stage III/IV means bone marrow failure, Binet stage B or Rai stage I/II means lots of solid disease but no bone marrow failure and Binet stage A or Rai stage 0 means no bone failure and little in the way of solid disease.

Clinical staging is very simple to perform and is a very powerful way of separating newly diagnosed patients into prognostic groups. However, most patients with newly diagnosed CLL have a low tumour burden. Although many of these early-stage patients will have a gentle form of CLL that has probably been around for years, others will have an aggressive form of the disease that just happens to have been caught early. Fortunately there are tests available that can identify patients with aggressive disease even if there is not much of it on board at the time of diagnosis.

Lymphocyte doubling time

This is really a reflection of how fast the CLL cells are dividing and is most useful in patients presenting with early-stage disease. Patients whose lymphocyte count takes less than 12 months to double have a poorer prognosis than those whose lymphocyte count takes more than 12 months to double. Some authorities consider that a lymphocyte doubling time of less than 6 months is an indication to start treatment. However, caution should be exercised as the lymphocyte count may fluctuate, sometimes dramatically, due to infection or stress.

Chromosomal abnormalities

Chromosomes are the structures that contain the cell's DNA or genetic blueprint. Each cell has 46 chromosomes consisting of two copies of chromosomes 1 to 22 and either two X chromosomes (females) or an X and Y chromosome (males). Each chromosome has a short arm called 'p' and a long arm called 'q'. Chromosomal abnormalities are very common in cancer as they frequently give rise to changes in the expression or function of molecules that result in increased cell growth or survival.

About three quarters of CLL patients have chromosomal abnormalities detectable by a technique called FISH (for fluorescence in-situ hybridisation). It should be stressed that these chromosomal abnormalities are almost always confined to the CLL cells and are not part of the patient's inherited genetic makeup. The four most common abnormalities in CLL are 17p-, 11q-, +12 and 13q-, where '-' means that a bit of the chromosome is missing (a deletion) and '+' means that there is an extra copy of the chromosome (a trisomy). Some patients have more than one of these abnormalities, in which case there is a 'pecking order' of importance (17p- then 11q- then +12 then 13q-). 13q- is said to be associated with a good-prognosis relative to having no chromosomal defects, +12 is said to be of neutral prognostic significance (although some studies suggest a negative effect), whereas 11q- and 17p- are bad, probably because they are often associated with disruption of an important cellular pathway that keeps cancer cells in check (see below). 17p- is especially bad.

p53 pathway abnormalities

p53 is a very important protein molecule that is activated when there is damage to the cell's DNA. Such damage happens spontaneously and can also be deliberately induced by chemotherapy or radiotherapy. Once activated, p53 stops the cell dividing so the damage can be repaired. If the damage is beyond repair, p53 triggers the cell to commit suicide by a process called apoptosis. In this way, p53 keeps cancer cells in check and also helps chemotherapy and radiotherapy to work. Conversely, cells in which p53 is not working properly are genetically unstable and are not killed effectively by radiation or chemotherapy. The gene encoding p53 is located on the short arm of chromosome 17 and is deleted in 17p- CLL.

To function properly, p53 requires another protein called ATM, which activates p53 in response to DNA damage. ATM is located on the long arm of chromosome 11 and is deleted in 11q- CLL. In



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some patients with CLL, p53 or ATM does not work properly because both copies of the gene are missing or defective. p53 pathway abnormalities can be detected in a number of ways, none of which is perfect. As already mentioned, chromosomal analysis by FISH (see above) can detect deletions of p53 (17p-) or ATM (11q-), although the p53 pathway may not always be defective in such cases. Alternatively, the p53 and ATM genes can be sequenced to look for mutations but this is quite difficult and time consuming. Because mutant p53 protein is usually present at increased levels, staining CLL cells for p53 protein over-expression can be used as a surrogate for p53 mutation analysis, although this is not always reliable. Finally, CLL cells can be tested in the laboratory to see whether p53 becomes activated when their DNA is damaged by radiation, but this test can be tricky to perform. p53 pathway abnormalities detected in each of these different ways have been linked to poor outcome in CLL.

IgV_H mutation and CD38

The purpose of normal B cells is to make antibodies to eliminate infection with bacteria, fungi or viruses. Each of the millions of B cells in the body has on its surface a unique 'receptor'. In this way, for every foreign structure or 'antigen' that could possibly exist, there is a B cell somewhere in the body that will recognise and bind to that structure. When a B-cell encounters its antigen, it is stimulated to proliferate, change into a 'plasma cell' and churn out large quantities of its receptor in the form of soluble antibody. The antibody then binds to its antigen, thereby coating the invading organism. A number of different mechanisms subsequently lead to the organism's destruction.

The tremendous variety in the B-cell receptor 'repertoire' is achieved because the receptor is made up of several building blocks, each of which is randomly selected from a large number of possible choices. In this way, there are millions of different combinations – a bit like a combination lock. Once a B cell has been stimulated to proliferate by antigen, the immune response can be fine-tuned by a process in which the daughter B cells can acquire random mutations in their receptor that change its structure slightly. This is called 'somatic hypermutation' and takes place in a part of the lymph node called the 'germinal centre'. If this results in an improved receptor that binds antigen more strongly, the B cells harbouring the modified receptor will undergo further rounds of proliferation. This process is called 'affinity maturation'. Mutations in the B-cell receptor can be measured by analysing the DNA sequence of part of the B-cell receptor called the IgV_H gene (short for variable region of the immunoglobulin heavy-chain gene). This is what is meant by 'IgV_H mutation'.

It has been known for some time that the amount of IgV_H mutation present in CLL clones varies between different patients. In 1999, groups in Bournemouth and New York simultaneously discovered that CLL patients with unmutated IgV_H genes had a significantly worse prognosis than those with mutated IgV_H genes. Both groups also found an association between unmutated IgV_H genes and the expression of a molecule called CD38 on the surface of CLL cells and showed that CD38+ cases had a worse prognosis than CD38- cases. These findings have been confirmed many times over by various groups around the world. Based on these findings, it was proposed that CLL was not one disease but two, IgV_H-mutated CLL being a relatively benign proliferation of CD38-negative 'post-germinal-centre' B cells and IgV_H-unmutated CLL a relatively aggressive proliferation of CD38-positive pre-germinal-centre B cells. Subsequent studies have shown that the two forms of CLL have much more in common than they have apart. Nevertheless, both IgV_H mutation status and CD38 expression remain very useful predictors of outcome in CLL.

V_H3-21

In 2001, a Swedish group noted that one of the B-cell receptor building blocks - the so-called V_H3-21 gene segment - is associated with a poor prognosis even though it is usually mutated.



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Other groups have confirmed these findings but have also noted that the amount of V_H mutation in CLL cells that use V_H3-21 is relatively low.

ZAP-70

Because sequencing IgV_H genes is quite difficult and time consuming, attempts were made to find other ways of identifying which patients had mutated versus unmutated IgV_H genes. To do this, both types of CLL were compared using a very powerful technique called 'gene expression profiling', which measures the expression of tens of thousands of genes all at the same time. A number of genes were found that were expressed differently in the two CLL groups. Amongst these, the one that was most consistently over-expressed in CLL cells with unmutated IgV_H genes was ZAP-70. ZAP-70 is a molecule normally expressed in T cells that helps them respond to antigen through their receptor (the latter having many similarities with the B-cell receptor). Subsequent studies showed that ZAP-70 protein could be detected relatively easily inside CLL cells by a routine technique called flow cytometry or FACS analysis and was associated with a poor prognosis. There is still debate concerning the best method to use for ZAP-70 measurement and how to set the cut-off between positivity and negativity.

Prognostic factors in clinical trials

Prognostic factors have been analysed for their ability to predict outcome in three clinical trials of first-line therapy. To cut a long story short, the most striking and consistent observation to come out these studies was that p53 deletion (17p-) was strongly associated with resistance to chemotherapy and short survival. In the UK study, it seemed to matter what proportion of CLL cells had a 17p deletion, 20% being the critical value. Other adverse prognostic factors such as 11q-, V_H3-21 usage and unmutated IgV_H genes seem to have less effect on response to chemotherapy but may influence long-term outcome.

Fortunately, p53 defects are not associated with therapeutic resistance to non-chemotherapy treatments such as alemtuzumab or high-dose methylprednisolone, and there is currently a UK study especially designed for patients with 17p- CLL in which these two agents are given in combination.

Discordance

Although all adverse prognostic factors overlap with one another to some extent, individual patients often have a mixture of good and bad prognostic features. Some attempts have been made to make sense of this. For example, it has been claimed that ZAP-70 negative patients with unmutated IgV_H genes often have 17p- or 11q-, whereas ZAP-70 positive patients with mutated IgV_H genes have a high frequency of V_H3-21 usage. Whether or not this is right, it seems that the adverse effect of different prognostic factors is probably additive, and that to predict outcome accurately requires more than one test and expert interpretation of the results.

Summary and practical advice

Although many different things can predict outcome in CLL, it should be stressed that there is as yet no proof that knowledge of these things influences outcome in any way. However, many patients find it useful to know how their disease is likely to behave. Prognostic profiling can also serve as a guide to overall therapeutic strategy and in particular help with transplant decisions

By virtue of having a blood count and being examined, all patients with CLL are automatically subjected the most basic and useful of prognostic tests, i.e. clinical staging. For patients with advanced-stage disease (especially Binet C or Rai III/IV), further prognostic tests, with the possible exception of FISH analysis for 17p-, are probably of limited value. For the rest, prognostic tests have the potential to predict how quickly the disease is likely to progress, how well chemotherapy is likely to work, and what sort of life span to expect. With regard to the latter,



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it should be borne in mind that some of the newer treatments may well prolong the natural history of CLL, although we currently have no proof that this is so.

For newly diagnosed patients requiring a broad picture of how their disease is likely to behave, a panel of prognostic tests is probably required including FISH for 17p-, 11q-, +12 and 13q-, and ideally at least two of the following: IgV_H status, CD38 or ZAP-70. These tests are not routinely funded by the NHS and are usually only available at centres that specialise in CLL. However, most hospitals should have links with such a centre. The tests are often performed as part of laboratory research projects that are done on the same blood sample. This is a good way of getting the tests done as it allows patients to benefit from the highest level of expertise and at the same time helps to take forward CLL research.

For patients whose CLL has already progressed to the point of requiring treatment, the most useful prognostic test is FISH analysis for 17p-. However, before looking for 17p- it is important to face up to the implications of finding it, namely that the prognosis is poor and that chemotherapy is very unlikely to work. On the other hand, having this information may help as there is currently a clinical trial (UKCLL06) specifically tailored for patients with 17p- CLL and designed to overcome some of the problems associated with p53 defects.

Separation of patients into prognostic groups will be an integral part of the next big UK trial of first-line therapy (CLL6). Based on data from the UK CLL4 trial, three patient risk groups have been identified: high risk (17p-), standard risk (unmutated IgV_H genes, V_H3-21 or 11q-), and low risk (none of these things). Patients entering CLL6 will have prognostic factors performed at trial entry and will enter different limbs of the trial according to what risk category they fall into. The trial will be one of the first to face up to the fact that CLL comes in different shapes and sizes and that a 'one-size-fits-all' approach may not be appropriate for many patients.

In summary, one of the most significant developments in CLL research in recent years has been to understand the disease's clinical variability in biological terms. This had led to the development of a number of tests that can predict disease progression, therapeutic response and survival. The next step will be to work out how best to use this information to guide therapeutic decisions in a way that will improve outcome. Clinical trials attempting to achieve this objective are already underway in the UK and more are to follow. Support for these studies is essential in order to take forward the treatment of CLL in a sensible and timely way.



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The following table is an overview of prognostic factors and their relevance.

Prognostic Indicator	Indolent	Intermediate	Aggressive
IgVH	Mutated		Un-mutated
ZAP 70	Less than 20% (-ve)		More than 20% (+ve)
CD38	Less than 30% (-ve)		More than 30% (+ve)
FISH	Normal karotype	Trisomy 12	11q deletion or 17p deletion
B2M	Less than 2.0		More than 4.0
Rai Staging	Stage 0 and I	Stage II	Stage III and IV
Binet Staging	Stage A	Stage B	Stage C
Lymphocyte Doubling Time	More than 12 months		Less than 12 months