The clinical significance of patients' sex in chronic lymphocytic leukemia

Daniel Catovsky,[1] Rachel Wade,[2] and Monica Else[1]

[1]Haemato-Oncology Research Unit, Division of Molecular Pathology, The Institute of Cancer Research, London; and [2]Clinical Trial Service Unit, Oxford, UK.

ABSTRACT

We examined the prognostic influence of gender in chronic lymphocytic leukemia. Data from four randomized trials (involving 1821 patients) and three registration studies of stage-A disease (involving 1299 patients) were analyzed. Overall survival at 10 years was better for women than men in all trials (27% *versus* 15%; *P*=0.0001) and in the registration series (55% *versus* 43%; *P*<0.0001). More women than men in the trials were Binet stage A-progressive (26% *versus* 15%), but gender was an independent predictor of survival in multivariate analysis of clinical variables (*P*<0.0001). Women responded better to treatment (overall response 83%) than men (71%; *P*<0.0001), within each stage and age group, although fewer women than men received the full treatment dose (79% *versus* 85%; *P*=0.01). Women were more likely than men to experience toxicity (85% *versus* 78%, *P*=0.01), particularly gastro-intestinal toxicity (57% *versus* 42%, *P*<0.0001). Laboratory markers in the LRF CLL4 trial showed a significantly lower incidence in women than men of unmutated *IGHV* genes, raised beta-2 microglobulin, CD38 and Zap-70 positivity and *TP53* deletions/mutations and/or 11q deletions. We also highlight the higher male:female ratios in randomized trials *versus* studies of early chronic lymphocytic leukemia and monoclonal B-cell lymphocytosis. Chronic lymphocytic leukemia in women runs a more benign clinical course than in men. Gender was also an independent predictor of response, suggesting that pharmacokinetic differences between the sexes and a possible effect of estrogens may contribute to the better outcome. Understanding the reasons for the different outcome by gender may improve patients' management. (*LRF CLL4 controlled-trials.com identifier: ISRCTN58585610*).

Introduction

The biological basis for the heterogeneity in clinical outcome of patients with chronic lymphocytic leukemia (CLL) has recently become better understood. Immunophenotypic, cytogenetic and molecular markers have emerged that help define prognosis more precisely.

The issue of gender in CLL was first explored by Rai *et al.* in 1975, in a small series of 125 patients.[1] It was noted that women had a better survival, although the difference was not found to be statistically significant after adjustment for age and stage. By 1989, an analysis of our first Medical Research Council CLL trial found that women did have a better overall survival, independently of age and tumor stage.[2] This study suggested a major difference between the sexes in the biology of CLL which has still not been widely recognized. Large population-based studies from Sweden[3] and the United States[4] subsequently confirmed the difference in overall survival between the sexes. Kristinsson *et al.*[3] documented a statistically significant inferior survival for men compared to women in all age groups and calendar periods in their 30-year study of patients with CLL. The updated SEER-18 Registry[4] also showed that women had a significantly better overall survival than men when the whole CLL population was evaluated and across different age groups, including when CLL-specific causes of death were considered separately.[4] The GIMEMA CLL group proposed a gender-based score for predicting clinical outcome of patients with early CLL (Binet stage A) based on their observation of a significantly better progression-free survival in females.[5]

The first indication of a biological basis for the differences in prognosis between the sexes came from studies on the mutational status of the immunoglobulin heavy chain variable region (*IGHV*) genes that showed that those with unmutated CLL[6,7] were predominantly males. Our own study of over 1000 cases, including familial and sporadic CLL,[8] showed that 37% of females had unmutated *IGHV* genes compared with 50% of males (*P*<0.0001).

In the present study, comprising two large series of patients, four randomized trials and three registration studies of stage-A CLL, we have further examined the influence of patients' sex on prognosis. Our observations may have implications for patients' management and may throw new light on the biology of this common leukemia.

Methods

We analyzed data from four randomized clinical trials that were conducted in the UK between 1978 and 2004 (Medical Research Council CLL1, 2 and 3 and LRF CLL4)[2,9-11] comprising 1821 previously untreated patients requiring treatment according to clinical criteria (Binet stages A progressive, B and C); all were evaluable for overall survival analysis, 1656 were evaluable for response to treatment, 1144 for toxicity (CLL3 and LRF CLL4) and 777 for progression-free survival (LRF CLL4 only) (Figure 1). In addition we examined data from 1299 patients with early CLL (Binet stage A) registered in three prospective studies between 1978 and 1998. A proportion of the latter (301 patients) were randomized to chlorambucil or no early therapy but for the purposes of this study were included in the control group (registration series). Details of the treatments used have been reported else-

©2014 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2013.101378
The online version of this article has a Supplementary Appendix.
Manuscript received on November 19, 2013. Manuscript accepted on March 18, 2014.
Correspondence: daniel.catovsky@icr.ac.uk

where.[2,9,10] Briefly, chlorambucil was one of the arms in every trial.[11] The comparators were the combination of COP (cyclophosphamide, oncovin and prednisolone) and splenic irradiation in CLL1, chlorambucil plus prednisolone and splenic irradiation in CLL2, chlorambucil plus epirubicin in CLL3, and fludarabine with or without cyclophosphamide in LRF CLL4. Biological, cytogenetic and molecular markers were available from patients entered in the LRF CLL4 trial and have been reported elsewhere, together with a full description of the cutoffs used to define positivity.[12,13] Causes of death were centrally categorized using the patients' death certificates and/or reports from the participating centers. Follow-up (overall survival only) was to June 2012 for the trials and June 2010 for the registration series, with a median follow-up for each trial of at least 9 years.

Statistical analyses used the chi square test for comparisons of incidence, response to therapy and toxicities. Kaplan-Meier survival curves were calculated and compared by the log-rank test. Overall survival was calculated from randomization to death from any cause. Progression-free survival in the LRF CLL4 trial was defined as the time from randomization to relapse needing further treatment, progression, or death from any cause. For non-responders and progressive disease, date of progression was when no response or progressive disease was recorded. Multivariate analyses of variables significantly associated with response and overall survival in univariate tests were performed by means of stepwise generalized linear modeling and the Cox proportional hazards model respectively. Values of $P \leq 0.05$ (two sided) were considered statistically significant. Analyses were performed using the STATISTICA software from StatSoft, a wholly owned subsidiary of Dell, Inc.

The LRF CLL4 trial was registered as an International Standard Randomized Trial, number ISRCTN58585610 and was approved by the UK multicenter research ethics committee (MREC). All four trials followed the UK Medical Research Council guidelines for good clinical practice. All patients provided informed consent. All authors had access to the primary clinical trial data. The main trial endpoints have been previously reported.[2,9,10]

Results

The main characteristics of the patients in both series are shown in Table 1. The proportion of females was significantly higher in the registration series than in the randomized series. Women were more likely than men to be aged ≥70 years in both series. They were more likely to have Binet stage A-progressive disease than stage B or C in the randomized series and slightly more likely to have Rai stage 0 disease than Rai stages I-II in the registration series.

Overall survival was significantly longer in women both in the randomized series [hazard ratio (HR): 0.72, 95% confidence interval (CI): 0.64–0.81, $P<0.0001$; Figure 2A] and in

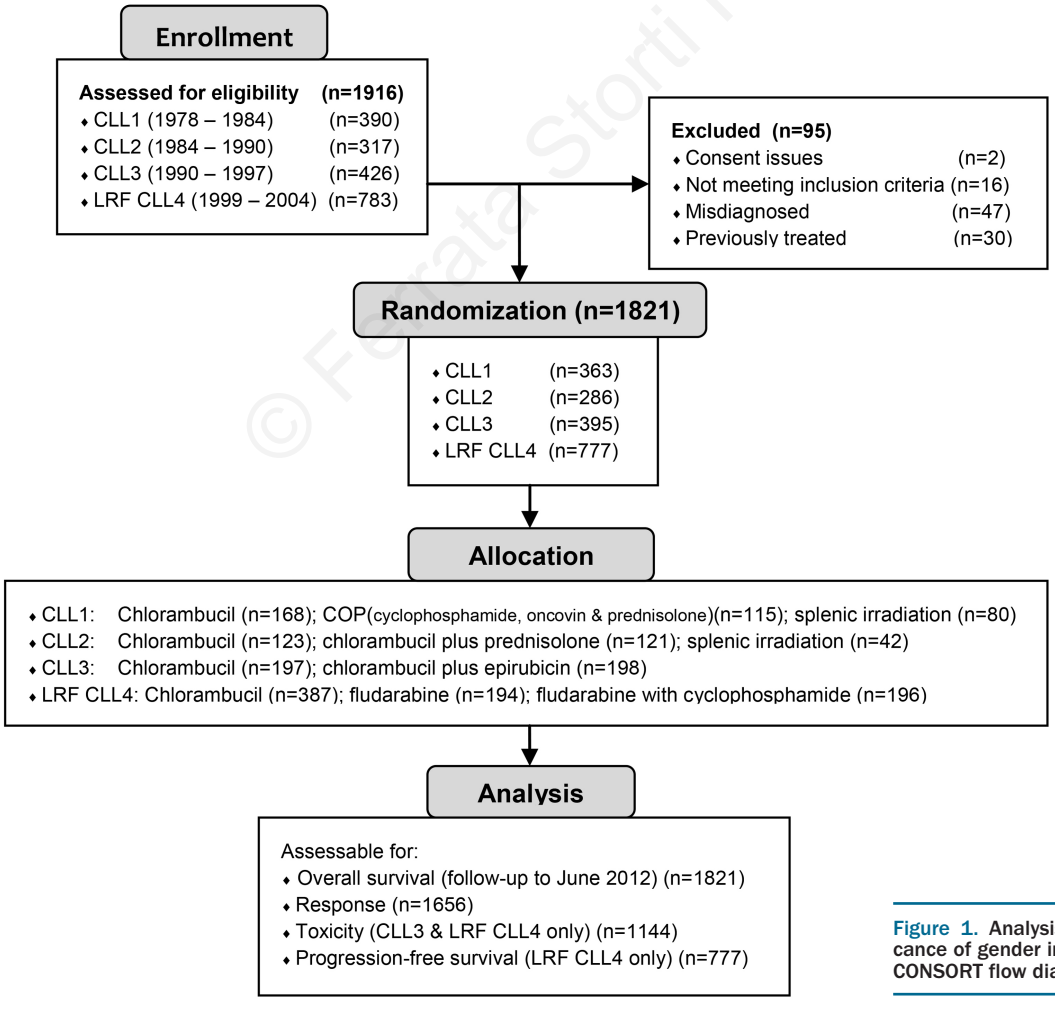


Figure 1. Analysis of the clinical significance of gender in four randomized trials: CONSORT flow diagram.

© Ferrata Storti Foundation

the registration series (HR: 0.71, 95% CI: 0.62–0.81, *P*<0.0001; Figure 2B). At 10 years, the overall survival rate in the randomized series was 27% (95% CI: 23–31%) for women and 15% (95% CI: 13–17%) for men. In the registration series it was 55% (95% CI: 51–59%) and 43% (95% CI: 39–47%), respectively. The overall survival rates at 10 years were better for women than for men in each of the four randomized trials although the difference did not quite reach statistical significance in CLL2 [CLL1: 23% (95% CI: 15–31%) *versus* 9% (95% CI: 6–13%), *Online Supplementary Figure S1A*; CLL2: 21% (95% CI: 12–30%) *versus* 11% (95% CI: 7–16%), *Online Supplementary Figure S1B*; CLL3: 20% (95% CI: 12–28%) *versus* 8% (95% CI: 5–11%), *Online Supplementary Figure S1C*; LRF CLL4: 35% (95% CI: 28–43%) *versus* 23% (95% CI: 19–27), *Online Supplementary Figure S1D*]. In the randomized trials 10-year overall survival rates were better for women than for men within each disease stage [stage A-progressive: 31% (95% CI: 22–39%) *versus* 23% (95% CI: 17–29%); stage B: 32% (95% CI: 24–39%) *versus* 16% (95% CI: 13–19%); stage C: 19% (95% CI: 13–25%) *versus* 11% (95% CI: 8–14%); *Online Supplementary Figure S2A*] and within each age group [<70 years: 33% (95% CI: 28–38%) *versus* 19% (95% CI: 17–22%); ≥70 years: 15% (95% CI: 9–21%) *versus* 4% (95% CI: 1–6%); *Online Supplementary Figure S2B*]. When patients aged <50 years were considered separately, in an attempt to investigate a possible effect of estrogens in women, the difference in overall survival rate at 10 years between women and men was similar to that seen in the other age groups studied [55% (95% CI: 36–75%) *versus* 31% (95% CI: 21–41%) respectively]. In the registration series women also had a better overall survival rate at 10 years than men within each Rai Stage and age group (*data not shown*).

Women were more likely than men to respond to treatment (Table 2) but were no more likely to attain a complete response. Response rates were 8%-14% higher in women in all stages, age groups, treatment categories and trials (Table 2). Response rates were no different between pre- and post-menopausal women (cutoff age 50 years; 85% *versus* 82% respectively; *P*=0.7). Women who responded to treatment had a better overall survival rate at 10 years than had male responders [31% (95% CI: 26–37%) *versus* 19% (95% CI: 16–22%)] but non-responders of both sexes had poor 10-year overall survival rates [14% (95% CI: 6–21%) *versus* 7% (95% CI: 4–10%); *Online Supplementary Figure S3A*]. Progression-free survival, assessed only in LRF CLL4

Table 1. Comparison of the two series by sex (3120 patients).

Variable		Randomized series Total	Male (%)	Female (%)	*P* value	Registration series * Total	Male (%)	Female (%)	*P* value
All		1821	1329 (73)	492 (27)		1299	772 (59)	527 (41)	<0.0001**
Age group (years)	<60	568	413 (31)	155 (31)		356	214 (28)	142 (27)	
	60-69	709	539 (41)	170 (35)	0.03	429	277 (36)	152 (29)	0.008
	70+	544	377 (28)	167 (34)		514	281 (36)	233 (44)	
Median age (years)			65	65			66.2	68.2	
Binet stage	A progressive	330	203 (15)	127 (26)					
	B	764	582 (44)	182 (37)	<0.0001				
	C	727	544 (41)	183 (37)					
Rai stage *	0					953	552 (71.5)	401 (76)	0.07
	I-II					346	220 (27.5)	126 (24)	

** All registration cases were Binet stage A; **P value for the comparison between the two series by the χ^2 test.*

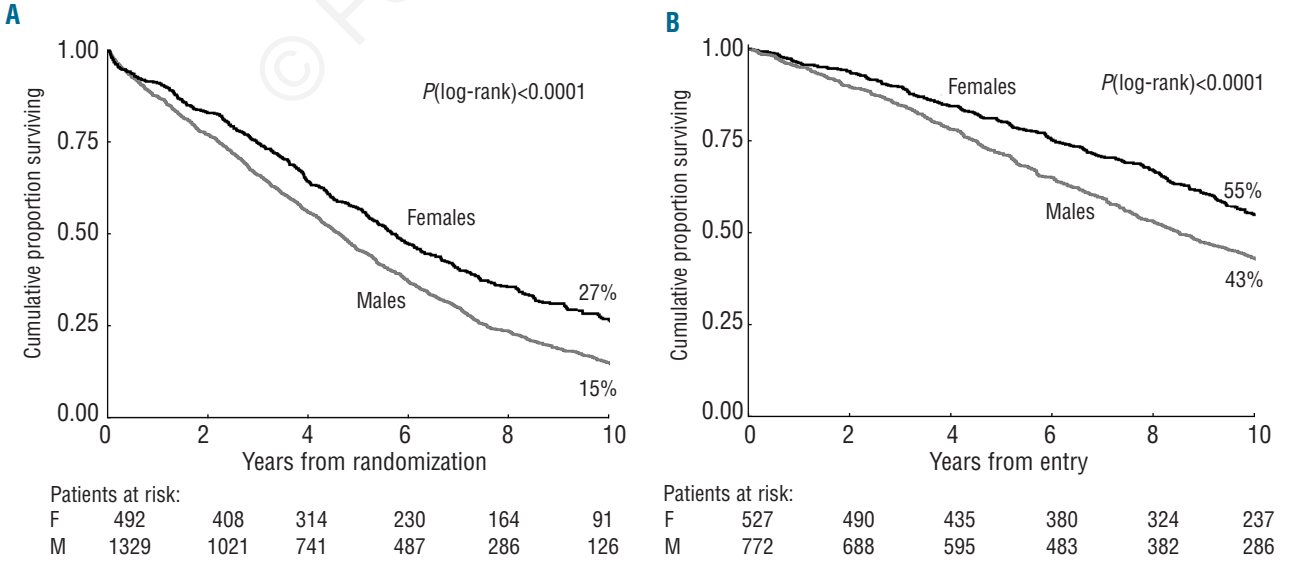


Figure 2. Overall survival by sex. (A) In all four randomized trials. (B) In the registration series.

© Ferrata Storti Foundation

and including non-responders to treatment, was longer for women (HR: 0.83, 95% CI: 0.70–0.98, *P*=0.03), though showing only a marginal difference at 3 years [38% (95% CI: 32–45%) for women *versus* 31% (95% CI: 27–35%) for men; *Online Supplementary Figure S3B*]. Data from the CLL3 and LRF CLL4 trials only showed that women were more likely to experience toxicity than men (85% *versus* 78%, *P*=0.01), particularly gastro-intestinal toxicity (nausea/vomiting and/or diarrhea; 57% *versus* 42%, *P*<0.0001; Table 3) and they were less likely than men to be given the full dose of treatment (79% *versus* 85%; *P*=0.01). Both sexes received the same number of treatment courses (median 6 courses, with 35% of women and 34% of men receiving fewer than 6 courses; data from CLL3 and LRF CLL4 trials only).

The causes of death were analyzed in the randomized series. These did not vary between the sexes. The respective proportions for females and males were as follows: CLL-related deaths: 79% *versus* 77% (Richter syndrome: 4% *versus* 3%); deaths unrelated to CLL: 21% *versus* 23% (cardiovascular: both 8%; other cancer: 10% *versus* 12%; other: both 3%). When non-CLL-related deaths were censored, overall survival remained significantly longer for women than for men (HR: 0.73, 95% CI: 0.64–0.84, *P*<0.0001).

Sex was an independent predictor of both response to treatment and overall survival in multivariate models which included variables available from patients in all four trials: sex, age, stage, white blood cell count, trial (LRF CLL4 *versus* earlier trials) and (for overall survival only) also response to treatment (Table 4). Biomarkers and molecular features were only available for multivariate analysis in the LRF CLL4 trial and the results, published elsewhere,[12-14] are not repeated here. A comparison of prognostic features according to sex is detailed in Table 5. Women had a significantly higher incidence than men of good prognostic features: low beta-2 microglobulin, mutated *IGHV* genes, absence of

Table 2. Overall response rate by sex (randomized trials; 1218 males and 438 females evaluable).

Variable	Male %	Female %	*P* value
All patients	71	83	<0.0001
- *Complete response*	16	19	0.5
Stage A progressive	77	88	0.02
Stage B	75	85	0.007
Stage C	64	76	0.006
Age <70 years	70	81	0.0005
Age ≥70 years	73	87	0.0008
Treatment			
Chlorambucil-based	69	83	<0.0001
Fludarabine-based	84	96	0.003
Other*	49	57	0.4
Trial			
CLL1	50	63	0.04
CLL2	73	84	0.08
CLL3	73	87	0.01
LRF CLL4	77	90	<0.0001

* *COP (cyclophosphamide, oncovin & prednisolone) or splenic irradiation.*

Table 3. Toxicity by sex in the CLL3 and LRF CLL4 trials.

Toxicity (all grades)	Males % n=849 *	Females % n=295 *	*P* value
A. Neutropenia	34	36	NS
B. Thrombocytopenia	15	12	NS
Any hematologic toxicity (A and/or B)	39	38	NS
C. Nausea & vomiting	38	50	0.0004
D. Diarrhea	13	18	0.05
Any gastro-intestinal toxicity (C and/or D)	42	57	<0.0001
E. Alopecia	23	26	NS
F. Other toxicity	37	38	NS
G. Febrile episodes	29	26	NS
H. Hospital admission	30	29	NS
Any toxicity (A – H)	78	85	0.01

* *Smallest number of assessable cases for any variable: males 772; females 260 (alopecia); NS: not significant*

Table 4. Multivariate analysis of prognostic factors found to be independent predictors of response to treatment and longer overall survival (randomized trials; 1656 evaluable patients).*

Response to treatment	Odds Ratio	95% CI	*P* value
Female sex	0.51	0.39-0.68	<0.0001
Stage A-progressive or B (*vs.* C)	0.65	0.52-0.82	0.0003
White blood count (<100x10^9/L)	0.74	0.59-0.93	0.009
LRF CLL4 trial (*vs.* earlier trials CLL1, 2 & 3)	0.60	0.48-0.76	<0.0001
Overall Survival	**Hazard Ratio**	**95% CI**	***P* value**
Female sex	0.69	0.61-0.78	<0.0001
Age <70 years	0.54	0.48-0.60	<0.0001
White blood count (<100x10^9/L)	0.80	0.72-0.89	<0.0001
LRF CLL4 trial (*vs.* CLL1, 2 & 3)	0.66	0.59-0.74	<0.0001
Response (*vs.* non-response) to treatment	0.55	0.49-0.62	<0.0001

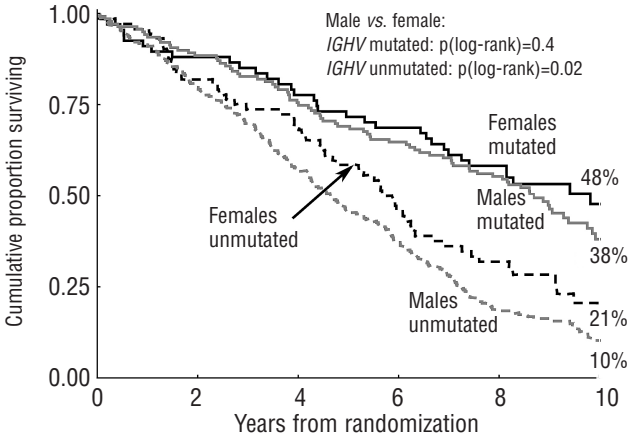
* *In these models age was not an independent predictor of response and disease stage was not an independent predictor of overall survival.*

Table 5. Incidence of laboratory findings by sex [male (M) *vs.* female (F)] in the LRF CLL4 trial.

Variable	Total evaluable M/F	M %	F %	*P* value
Beta2 microglobulin ≥4 mg/L	410/146	48	35	0.007
Unmutated *IGHV* genes (98% cut off)	394/139	65	52	0.007
TP53 deletion (10% cutoff) and/or mutation	432/148	9	7	0.7
11q deletion (5% cutoff)	430/149	22	15	0.06
TP53 del/mut and/or 11q del	430/148	30	20	0.01
Trisomy 12 (3% cutoff)	430/149	16	15	0.9
CD38 positive (7% cutoff)	398/137	67	50	0.0003
ZAP-70 positive (10% cutoff)	358/120	52	42	0.05
13q deletion (5% cutoff)	430/149	59	63	0.3
Notch1 mutation	344/122	11	7	0.3
SF3B1 mutation	319/118	18	14	0.4
CLLU1 positive	378/137	54	47	0.2

© Ferrata Storti Foundation

TP53 abnormalities and/or 11q deletion, and CD38 and Zap-70 negativity. In patients with unmutated *IGHV* genes, overall survival was significantly longer in women (HR: 0.72, 95% CI: 0.54–0.97, *P*=0.03), with 10-year overall survival rates of 21% (95% CI: 10–31%) for women and 10% (95% CI: 6–15%) for men (Figure 3). In patients with mutated *IGHV* genes, overall survival was marginally, but not significantly, better for women, with 10-year overall survival rates of 48% (95% CI: 35–61%) for women *versus* 38% (95% CI: 29–47%) for men.



Patients at risk:

Mutated:						
F	67	59	52	46	38	17
M	139	123	105	90	73	25
Unmutated:						
F	72	59	50	33	21	6
M	255	203	145	96	42	14

Figure 3. Overall survival by sex and *IGHV* mutation status in the LRF CLL4 trial.

Discussion

This study demonstrates that CLL runs a more benign clinical course in women than in men. This is shown by the consistently better overall survival in our four randomized trials and in our registration control series of early CLL, as well as in large population-based studies.[3,4] Further support for this finding is seen in women's longer progression-free survival, shown here in our LRF CLL4 trial and also in an Italian series of patients with Binet stage A CLL.[5] In addition, we report that women had a better overall response to treatment than men (Table 2) and greater gastro-intestinal toxicity. These two findings, to our knowledge, have not been previously reported in CLL. We suggest that it may be important to examine outcomes by sex in recent and/or current randomized trials, including in particular progression-free survival, which we could only examine in LRF CLL4.

No good hypotheses have been advanced to explain the observed trend for a better outcome in women.[2-5] We have identified three possible factors which may contribute to the better treatment response and longer survival in women. Firstly, the association with good prognostic factors; secondly, pharmacokinetic differences between the sexes; and thirdly the effect of estrogens.

A major contributing factor to the better outcome in women is the strong association with good prognostic factors, chiefly mutated *IGHV* genes, as a consequence of as yet unidentified mechanisms. Although our initial report from the Medical Research Council CLL1 trial showed that the longer overall survival in women was independent of stage and age[2] this observation was still considered probably a reflection of the longer overall survival of women in the general population. However our finding is now underpinned by biological features of good prognosis in females, including a significantly higher incidence of mutated *IGHV* genes,[6-8] lower expression of CD38 and ZAP-70, lower beta-2 microglobulin levels and fewer cytogenetic abnormalities such as *TP53* deletion/mutation and/or 11q deletion (Table 5). A recent report of genotypic male:female ratios in 4698 patients found that in patients with 11q del, alone or with other abnormalities, the male:female ratio (2.5) was significantly higher than in patients with trisomy 12, 13q del or 17p del (male:female ratio ~1.5).[15] Why there is a different incidence of prognostic factors between men and women is not known, but may be related to genetic factors determining predisposition. An opportunity for further study is possible, as at least 30 loci of common gene variants have now been identified as predisposing to CLL.[16]

In an early report from the LRF CLL4 trial, including the above biomarkers, male sex was associated with shorter overall survival in univariate analysis.[12] More recently, both in a second analysis of the LRF CLL4 trial, with a further 4 years of follow-up,[13] and also in another study of 1160 patients,[17] male sex was found to be an independent predictor of shorter overall survival. Both these analyses included *NOTCH1* and *SF3B1* gene mutations. It would, therefore, appear that the prevalence of good prognostic factors in women does not wholly account for their better survival and that there may be other reasons for the better outcomes in women, beyond the known prognostic factors. Evidence to support this view is provided by our finding that, even when women had unmutated *IGHV* genes, they had longer overall survival than their male counterparts.

Gender variation in drug efficacy and toxicity is increasingly being reported, particularly gender differences in pharmacokinetics.[18] Hepatic clearance of drugs is a function of hepatic enzymes such as the cytochrome (CYP) P450 system, which is responsible for the metabolism of many drugs. Hepatic CYP2B6, whose substrates include the alkylating agents cyclophosphamide and ifosfamide, is expressed differently between the sexes.[19] Significantly higher amounts of CYP2B6 mRNA protein and enzyme activity were found in females than in males. As a consequence some agents will be more effective in females and may, at the same time, show more toxicity.[18,19] It is of interest that in our trials women responded significantly better than men (Table 2) although they experienced more toxicity, particularly gastro-intestinal toxicity, and were thus less likely to have received the full dose of treatment.

Although our trials did not include treatment with monoclonal antibodies, recent pharmacokinetic data showed that the clearance of rituximab is reduced and its elimination half-life more prolonged in women than in men.[20] Higher rituximab serum concentrations before next therapy and higher response rates were associated, in another pharmacokinetic study,[21] with female sex. It therefore seems relevant that our reported observations should also be examined in trials with the combination of fludarabine, cyclophosphamide and rituximab in CLL.[22,23] In fact a recent

©Ferrata Storti Foundation

Table 6. Male:female (M:F) ratios in CLL trials, early CLL and monoclonal B-cell lymphocytosis.

Author	Monoclonal B-cell Lymphocytosis N. of cases	M:F ratio	Early CLL (stage A) N. of cases	M:F ratio	Treatment Trials N. of cases	M:F ratio
Rawstron *et al.*, 2008[29]	387	0.9:1 *	-	-	-	-
Shanafelt *et al.*, 2009[30]	302	1.4:1	94 (Rai 0)**	1.6:1	-	-
Rossi *et al.*, 2009[31]	123	1.0:1	154 (Rai 0)	1.2:1	-	-
Scarfò *et al.*, 2012[32]	184	1.1:1	430 (Rai 0)	1.2:1	-	-
Molica *et al.*, 2005[5]	-	-	1138 (Binet A)	1.2:1	-	-
This series	**-**	**-**	**1299 (Binet A)**	**1.5:1**	**1821**	**2.7:1**
Tam *et al.*, 2008[22]	-	-	-	-	300	2.3:1
Flinn *et al.*, 2007[33]	-	-	-	-	278	2.3:1
Hillmen *et al.*, 2007[34]	-	-	-	-	297	2.5:1
Eichhorst *et al.*, 2006[35]	-	-	-	-	362	2.7:1
Sutton *et al.*, 2011[36]	-	-	-	-	241	3.0:1
Woyach *et al.*, 2011[37]	-	-	-	-	104	3.3:1
Hallek *et al.*, 2010[23]	-	-	-	-	817	2.8:1

*Subjects with a normal white blood count M:F ratio 0.71:1; in those with lymphocyte count ≥ $4x10^9$/L M:F ratio 0.93:1. **lymphocyte count ≤ $10x10^9$/L.

large retrospective study from registries of patients with CLL aged 66 years or older, comparing rituximab plus chemotherapy with chemotherapy alone, highlighted male gender as an independent factor associated with shorter overall survival.[24]

In our study we attempted to examine a possible effect of estrogens by analyzing differences in survival and response using a cutoff at 50 years of age, this being the approximate age of women at menopause. Although we were unable to find a difference, it is still possible that estrogens may have an impact on the different outcomes for men and women with CLL. Firstly, it has been shown that there may be an inhibitory effect of estrogens on the activity of superoxide dismutases, essential enzymes that protect cells from damage induced by free radicals in experiments using CLL cells.[25] Secondly, it has been suggested that females may have a more effective immune response than males as a result of estrogen-mediated inhibition of caspase-12 expression.[26] We suggest that both these mechanisms may be operational, protecting against infections and also contributing to women's better response to treatment through either improved cytotoxicity or enhanced immune function.

It is of interest that female sex has emerged recently as a predictor of better outcomes not only in CLL but also, for example, in cutaneous head and neck melanoma.[27] Another recent report, in chronic myeloid leukemia, included female sex as one of the two more significant predictors of stable molecular response after long-term imatinib therapy.[28]

Another observation in our study is that the proportion of males is higher in patients requiring treatment than in patients with earlier stage disease and hence is reflected in most clinical trials (Table 6). As in our registration series, the male:female ratios are lower in observational series of Binet stage A or Rai stage 0. Furthermore, the male:female ratio is even lower in monoclonal B-cell lymphocytosis, a precursor condition of CLL (Table 6). This is a novel observation not reported before and it is probably the strongest evidence that the disease course in women is slower and/or more benign than in men. The disease appears to begin equally in both sexes from the starting point of monoclonal B-cell lymphocytosis. Thereafter CLL in men becomes gradually more represented in observational series and doubles at the stage of symptomatic progressive disease, as seen in all clinical trials. The higher proportion of *IGHV* unmutated cases in men may be a factor, as this characteristic has been shown to be associated with progressive disease in all studies thus far.

While the implications of gender differences in the pathogenesis of CLL and its treatment require further studies, it should be recognized that CLL has a more benign clinical course in women than in men. The observed gender differences may bring about some changes in treatment modalities. In particular, women may require modifications in the dosage of the more intensive therapies, as it appears that the thresholds for both efficacy and gastro-intestinal toxicity may be lower in women. The increase in the male:female ratio from monoclonal B-cell lymphocytosis, through early stage CLL, to CLL requiring treatment shows that the more active, aggressive forms of CLL are seen predominantly in men and new therapeutic targets should focus on that finding.

Acknowledgments

The authors would like to thank David Gonzalez, Alison Morilla, David Oscier and Jonathan Strefford who performed the laboratory studies in the LRF CLL4 trial. We also thank Professors Martin Dyer, Mel Greaves, Richard Houlston and David Oscier for their editorial advice. We are particularly grateful to Dr Sue Richards, the chief statistician of the reported CLL trials, for her painstaking work and dedication over the last 35 years. The early CLL trials (1 – 3) were funded by the Medical Research Council. The LRF CLL4 trial was funded by a core grant from Leukaemia and Lymphoma Research. The CTSU received research support from the Medical Research Council and Cancer Research UK. ME was supported by the Arbib Foundation. Laboratory studies in the LRF CLL4 trial were supported by an educational grant from Schering Health Care (UK) and Schering AG (Germany).

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46(2):219-34.
2. Catovsky D, Fooks J, Richards S. Prognostic factors in chronic lymphocytic leukaemia: the importance of age, sex and response to treatment in survival. A report from the MRC CLL 1 trial. MRC Working Party on Leukaemia in Adults. Br J Haematol. 1989;72(2):141-9.
3. Kristinsson SY, Dickman PW, Wilson WH, Caporaso N, Björkholm M, Landgren O. Improved survival in chronic lymphocytic leukemia in the past decade: a population-based study including 11,179 patients diagnosed between 1973-2003 in Sweden. Haematologica. 2009;94(9):1259-65.
4. Ailawadhi S, Yang D, Jain N, Mie Thinn M, Cozen W, Chanan-Khan A. Ethnic disparities in chronic lymphocytic leukemia survival: a SEER database review. Blood. 2012;120(21):757. Abstract 757.
5. Molica S, Mauro FR, Callea V, Gentile M, Giannarelli D, Lopez M, et al; GIMEMA CLL Study Group. A gender-based score system predicts the clinical outcome of patients with early B-cell chronic lymphocytic leukemia. Leuk Lymphoma. 2005;46(4):553-60.
6. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840-7.
7. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood. 2002;100(4):1177-84.
8. Crowther-Swanepoel D, Wild R, Sellick G, Dyer MJ, Mauro FR, Cuthbert RJ, et al. Insight into the pathogenesis of chronic lymphocytic leukemia (CLL) through analysis of IgVH gene usage and mutation status in familial CLL. Blood. 2008;111(12):5691-3.
9. Catovsky D, Richards S, Fooks J, Hamblin TJ. CLL trials in the United Kingdom: the Medical Research Council trials 1, 2 and 3. Leuk Lymphoma. 1991;5(Suppl1):105-11.
10. Catovsky D, Richards S, Matutes E, Oscier D, Dyer MJ, Bezares RF,et al; UK National Cancer Research Institute (NCRI) Haematological Oncology Clinical Studies Group; NCRI Chronic Lymphocytic Leukaemia Working Group. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 trial): a randomised controlled trial. Lancet. 2007;370 (9583):230-9.
11. Catovsky D, Else M, Richards S. Chlorambucil--still not bad: a reappraisal. Clin Lymphoma Myeloma Leuk. 2011;11 (Suppl 1):S2-6.
12. Oscier D, Wade R, Davis Z, Morilla A, Best G, Richards S, et al; Chronic Lymphocytic Leukaemia Working Group, UK National Cancer Research Institute. Prognostic factors identified three risk groups in the LRF CLL4 trial, independent of treatment allocation. Haematologica. 2010;95(10):1705-12.
13. Oscier DG, Rose-Zerilli MJ, Winkelmann N, Gonzalez de Castro D, Gomez B, Forster J, et al. The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. Blood. 2013;121(3):468-75.
14. Gonzalez D, Else M, Wren D, Usai M, Buhl AM, Parker A, et al. CLLU1 expression has prognostic value in chronic lymphocytic leukemia after first-line therapy in younger patients and in those with mutated IGHV genes. Haematologica. 2013;98(2):274-8.
15. Cantú ES, McGill JR, Stephenson CF, Hoffmann HM, Tang L, Yan J, et al. Male-to-female sex ratios of abnormalities detected by fluorescence in situ hybridization in a population of chronic lymphocytic leukemia patients. Hematol Rep. 2013;5(1):13-7.
16. Speedy HE, Di Bernardo MC, Sava GP, Dyer MJ, Holroyd A, Wang Y, et al. A genome-wide association study identifies multiple susceptibility loci for chronic lymphocytic leukemia. Nat Genet. 2014;46(1):56-60.
17. Jeromin S, Weissmann S, Haferlach C, Dicker F, Bayer K, Grossmann V, et al. SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia. 2014;28(1):108-17.
18. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol. 2004;44:499-523.
19. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther. 2003;307(3):906-22.
20. Müller C, Murawski N, Wiesen MH, Held G, Poeschel V, Zeynalova S, et al. The role of sex and weight on rituximab clearance and serum elimination half-life in elderly patients with DLBCL. Blood. 2012;119(14): 3276-84.
21. Jäger U, Fridrik M, Zeitlinger M, Heintel D, Hopfinger G, Burgstaller S, et al; Arbeitsgemeinschaft Medikamentöse Tumortherapie (AGMT) Investigators. Rituximab serum concentrations during immuno-chemotherapy of follicular lymphoma correlate with patient gender, bone marrow infiltration and clinical response. Haematologica. 2012;97(9):1431-8.
22. Tam CS, O'Brien S, Wierda W, Kantarjian H, Wen S, Do KA, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood. 2008;112(4):975-80.
23. Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, et al; International Group of Investigators; German Chronic Lymphocytic Leukaemia Study Group. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010;376(9747):1164-74.
24. Danese MD, Griffiths RI, Gleeson M, Satram-Hoang S, Knopf K, Mikhael J, et al. An observational study of outcomes after initial infused therapy in Medicare patients diagnosed with chronic lymphocytic leukemia. Blood. 2011;117(13):3505-13.
25. Huang P, Feng L, Oldham EA, Keating MJ, Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. Nature. 2000;407(6802):390-5.
26. Yeretssian G, Doiron K, Shao W, Leavitt BR, Hayden MR, Nicholson DW, et al. Gender differences in expression of the human caspase-12 long variant determines susceptibility to Listeria monocytogenes infection. Proc Natl Acad Sci USA. 2009; 106(22):9016-20.
27. Arce PM, Camilon PR, Stokes WA, Nguyen SA, Lentsch EJ. Is sex an independent prognostic factor in cutaneous head and neck melanoma? Laryngoscope. 2013 Oct 1. doi: 10.1002/lary.24439. [Epub ahead of print].
28. Branford S, Yeung DT, Ross DM, Prime JA, Field CR, Altamura HK, et al. Early molecular response and female sex strongly predict stable undetectable BCR-ABL1, the criteria for imatinib discontinuation in patients with CML. Blood. 2013;121(19):3818-24.
29. Rawstron AC, Bennett FL, O'Connor SJ, Kwok M, Fenton JA, Plummer M, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008;359(6):575-83.
30. Shanafelt TD, Kay NE, Rabe KG, Call TG, Zent CS, Maddocks K, et al. Brief report: natural history of individuals with clinically recognized monoclonal B-cell lymphocytosis compared with patients with Rai 0 chronic lymphocytic leukemia. J Clin Oncol. 2009;27(24):3959-63.
31. Rossi D, Sozzi E, Puma A, De Paoli L, Rasi S, Spina V, et al. The prognosis of clinical monoclonal B cell lymphocytosis differs from prognosis of Rai 0 chronic lymphocytic leukaemia and is recapitulated by biological risk factors. Br J Haematol. 2009;146(1):64-75.
32. Scarfò L, Zibellini S, Tedeschi A, Maura F, Neri A, Bertazzoni P, et al. Impact of B-cell count and imaging screening in cMBL: any need to revise the current guidelines? Leukemia. 2012;26(7):1703-7.
33. Flinn IW, Neuberg DS, Grever MR, Dewald GW, Bennett JM, Paietta EM, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol. 2007;25(7):793-8.
34. Hillmen P, Skotnicki AB, Robak T, Jaksic B, Dmoszynska A, Wu J, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol. 2007;25(35):5616-23.
35. Eichhorst BF, Busch R, Hopfinger G, Pasold R, Hensel M, Steinbrecher C, et al; German CLL Study Group. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood. 2006; 107(3):885-91.
36. Sutton L, Chevret S, Tournilhac O, Diviné M, Leblond V, Corront B, et al; Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) and Groupe Français d'étude de la Leucémie Lymphoïde Chronique (GFLLC). Autologous stem cell transplantation as a first-line treatment strategy for chronic lymphocytic leukemia: a multicenter, randomized, controlled trial from the SFGM-TC and GFLLC. Blood. 2011;117(23):6109-19.
37. Woyach JA, Ruppert AS, Heerema NA, Peterson BL, Gribben JG, Morrison VA, et al. Chemoimmunotherapy with fludarabine and rituximab produces extended overall survival and progression-free survival in chronic lymphocytic leukemia: long-term follow-up of CALGB study 9712. J Clin Oncol. 2011;29(10):1349-55.

©Ferrata Storti Foundation