Lymphoma in patients treated with anti-TNF: results of the 3-year prospective French RATIO registry.

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Abstract

Objective: To describe cases of lymphoma associated with anti-TNF therapy, identify risk factors, estimate the incidence and compare risks for different anti-TNF agents.

Methods: We designed a national prospective registry (RATIO) from 2004 to 2006, for collecting all cases of lymphoma in French patients receiving anti-TNF therapy, whatever the indication. We conducted a case-control analysis including two controls treated with anti-TNF per case and an incidence study of lymphoma with the French population used as reference. .

Results: We collected 38 cases of lymphoma, 31 non-Hodgkin's lymphoma (NHL) (26 B-cell and 5 T-cell), 5 Hodgkin's lymphoma (HL) and 2 Hodgkin's-like lymphoma. Epstein-Barr virus (EBV) was detected in 2 of 2 Hodgkin's-like lymphoma, 3 of 5 HL and one NHL. Patients receiving adalimumab or infliximab had a higher risk than those treated with etanercept: SIR = 4.1 (2.3–7.1) and 3.6 (2.3–5.6) versus 0.9 (0.4–1.8). The exposure to adalimumab or infliximab versus etanercept was an independent risk factor for lymphoma in the case-control study: odds ratio=4.7 (1.3–17.7) and 4.1 (1.4–12.5), respectively. The sex and age- adjusted incidence rate of lymphoma was 42.1 per 100,000 patient-years. The standardized incidence ratio (SIR) was 2.4 (95% confidence interval [CI] 1.7–3.2).

Conclusion: Some lymphomas associated with immunosuppression may occur in patients receiving anti TNF therapy, and the risk of lymphoma is higher with monoclonal-antibody therapy than with soluble-receptor therapy.

INTRODUCTION

The risk of lymphoma is increased in several systemic autoimmune diseases, mainly Sjögren's syndrome, systemic lupus erythematosus and rheumatoid arthritis (RA)[1]. In RA, the risk of non-Hodgkin's lymphoma (NHL) is increased by twofold[2] and that of Hodgkin's lymphoma (HL) by threefold.[3] Long-lasting inflammatory activity of RA is considered the main risk factor of lymphoma by its continuous stimulation of B-cells.[4]

The effect of immunosuppressive drugs on the risk of lymphoma remains a matter of debate. To date, only the deleterious role of azathioprine has been demonstrated for both RA][4] and Crohn's disease (CD).[5] Although withdrawal of methotrexate (MTX) treatment can rarely induce regression of Epstein-Barr virus (EBV)-associated lymphoproliferation,[6] most recent reports did not find any increased risk of NHL in RA patients treated with MTX.[7 8]

Recent concerns about lymphoma have focused on therapy with anti-TNF drugs because of their profound immunoregulatory effect. However, anti-TNF therapy could reduce the inflammatory activity of the underlying disease, which is the main risk factor for lymphoma in RA.

In some cohorts of RA patients receiving anti-TNF therapy, the risk of lymphoma was not different than that for RA patients not receiving the therapy.[7 9-11] However, these cohort studies were underpowered to investigate a difference between anti-TNF agents in terms of risk of lymphoma.

We aimed to examine whether patients receiving anti-TNF agents have an increased lymphoma risk and to compare risks for different anti-TNF agents, described the cases of lymphoma and their outcome, and identified the risk factors of lymphoma in patients receiving anti-TNF therapy.

PATIENTS AND METHODS

The French RATIO (Research Axed on Tolerance of bIOtherapies) registry was designed by a multidisciplinary group to prospectively collect all cases of lymphoma occurring in France from February 1, 2004, to January 31, 2007, in patients who were receiving anti-TNF therapy, for whatever the indication. The design has been described elsewhere.[12 13] The reporting of this study conforms to the STROBE statement.[14]

Identification and validation of lymphoma cases

All cases reported to the 31 French pharmaco-vigilance regional centers of Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), or pointed out directly to the companies commercializing anti-TNF were collected. In addition, physicians from all the different French hospital centers implied either in the prescription of TNF blockers (i.e. rheumatology, internal medicine, gastroenterology and dermatology departments) and/or in the management of lymphomas (i.e. hematology or oncology centers), were directly required to report each newly diagnosed case). A direct mail reminder 4 times a year and several communications at congresses or in specialized press encouraged them to report cases.

Validation of cases: Included in the RATIO registry were all cases (from all sources) with a validated diagnosis of lymphoma according to the International Classification of Diseases for Oncology (categories 9590-9599, 9650-9660, 9670-9680, 9690-9699, 9700-9709, 9710-9719). An expert committee involving 3 experts of lymphoma (XM, OH, MR) validated cases by consensus on the basis of the detailed standardized case report form and additional documents (hospitalization summary, histological results or others). The biopsy specimens of all validated cases were reviewed by the same hematopathologist (MR), to validate the diagnosis obtained by histopathology. In addition, this hematopathologist assessed all biopsy specimens for presence of EBV, detected by Eber *in situ* hybridization.

Risk of lymphoma for patients receiving anti-TNF therapy

A case-control study was performed..

Cases: Cases were all validated cases of lymphoma in the RATIO registry with a labeling indication for use of anti-TNF treatment (i.e. RA, spondylarthropathy [SpA; AS or psoriatic arthritis], UC or CD, or psoriasis).

Controls: Lymphoma-free patients receiving anti-TNF treatment in a labeling indication were included from centers participating in the RATIO registry (thus from the same population source) in a global pool of controls. From that pool, we randomly selected patients for a database of controls reflecting the proportion of patients

receiving each of the three anti-TNF drug in France. Two controls per case were randomly matched by sex, age (within 5 years) and underlying inflammatory disease from this database of controls. We also used a second sample of controls randomly selected from the same database of controls, with the same matching criteria (second matching).

Incidence study

Incidence of lymphoma

We estimated the annual incidence rate of lymphoma in patients treated with anti-TNF therapy, adjusted for age and sex, with the French population as a reference (see supplementary file for details).

Statistical analysis

The number of cases of lymphoma in France during the study period determined the sample size. A descriptive analysis was performed for the whole sample. We identified the risk factors of lymphoma by both univariate and multivariate analysis (conditional logistic regression model). The SIR was calculated for anti-TNF agents use as a whole and for agents used individually. We performed subgroup and sensitivity analyses. (see supplementary file for details).

Compliance with research ethics standards

This study was authorized by the ethic committee of AP-HP, GHU Nord (Institutional Review Board of Paris North Hospitals, Paris 7 University, AP-HP; authorization number 162-08). The registry was reported at clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT00224562).

RESULTS

Description of the cases

We collected data on 41 cases of lymphomas, and 38 cases were validated. Among them, 31 were NHL (26 B-cell and 5 T-cell), 5 HL and 2 Hodgkin's-like lymphoma. The characteristics of the cases are in Table 1.

Biopsy specimens were reviewed for 36 cases (29 NHL, 5 HL and 2 Hodgkin's-like lymphomas). EBV was detected in 2 of 2 Hodgkin's-like lymphoma, 3 of 5 HL and one B-cell lymphoproliferation.

The underlying disease was RA in 27 cases, SpA in 7 cases (AS in 4 cases and psoriatic arthritis in 3 cases), CD in 3 cases and primary Sjögren's syndrome in one case. Secondary Sjögren's syndrome was present in 3 of 27 patients with RA and lymphoma. All patients were HIV negative. Most patients (31/38) had received only one anti-TNF agent. The 3 patients with CD and lymphoma had previously received azathioprine.

Outcome

Three patients with low-grade NHL received no lymphoma-specific treatment, and anti-TNF therapy was stopped. They remained with stable disease, without any progression or regression of the lymphoma (follow-up 19.8-37.0 months). Among the other patients, 29 received chemotherapy, 3 rituximab alone, and 2 radiotherapy, and 1 died before receiving chemotherapy. At last follow-up (median follow-up 18.2 months), 16 cases were in remission, in 3 disease was stable without specific treatment of the lymphoma, in 3 disease relapsed, 7 were still being treated and 9 patients died (24%); 4 of 27 with B-cell NHL, 2 of 5 with T-cell NHL and 3 of 7 with HL and Hodgkin's-like lymphoma.

Time occurrence of lymphoma with anti-TNF therapy

The median time from onset of anti-TNF treatment and the first symptoms of lymphoma was 23.6 months. In 5 patients, lymphoma occurred, but anti-TNF therapy had been discontinued 6.1 to 44.1 months before. For these 5 patients, the last anti-TNF agent received was infliximab for 3 and adalimumab for 2. As indicated in Figure 1, the relation between cumulative frequency of lymphoma and time from onset of anti-TNF therapy appeared to be approximately linear for the first or last anti-TNF agent received and did not differ by drug received.

Risk factors of lymphoma for patients receiving anti-TNF therapy

The case-control study involved 37 cases and 74 controls (as described in the methods, the patient treated for Sjögren's syndrome was not included). The repartition of the 3 anti-TNF in the control group corresponded to that found in the

country during the 2004-2006 period, with 18% receiving adalimumab, 51% etanercept and 31% infliximab. The results of univariate analysis are given in Table 2.

On the multivariate analysis (Table 3), 2 factors were independently associated with occurrence of lymphoma in patients receiving anti-TNF therapy: anti-TNF treatment duration less than 2 years (OR=3.30 [1.17–9.30]) and treatment with infliximab or adalimumab versus etanercept (OR=4.12 [1.36–12.49] and OR=4.73 [1.27–17.65], respectively). In the case-control study restricted to RA cases, only one factor was associated with occurrence of lymphoma: treatment with infliximab or adalimumab versus etanercept (OR=6.68 [1.90–23.54]). The ORs of adalimumab or infliximab versus etanercept were also very similar with the second matching and in subgroup and sensitivity analyses (Figure 2).

Incidence and risk of lymphoma for patients receiving anti-TNF therapy compared with the general population

The main analysis relied on a total number of 57,711 patient-years of use of anti-TNF therapy during the 2004-2006 period, as the denominator of the incidence rate. The annual incidence rate of lymphoma adjusted for age and sex among patients receiving anti-TNF therapy, with the French population as a reference, was 42.1 (95% CI 6.9-77.2 per 100,000 person-years). The SIR was 2.4 (95% CI 1.7–3.2; p<0.0001) (Figure 3). For RA and SpA, the SIR was 2.3 (1.6–3.3; p<0.0001) and 1.9 (0.9–4.0; p=0.09), respectively.

Like for the case-control study, the incidence of lymphoma for patients receiving anti-TNF therapy differed depending on the agent received. The incidence rates for patients receiving etanercept, adalimumab, and infliximab were 15.3 (95% CI 0.0-45.6) per 100,000 person-years, 65.1 (95% CI 0.0-160.0) per 100,000 person-years and 69.1 (95% CI 0.0-150.4) per 100,000 person-years, respectively. The SIRs were 0.9 (0.4–1.8; p=0.72), 4.1 (2.3–7.1; p<0.0001), and 3.6 (2.3–5.6; p<0.0001), respectively. We found a difference between etanercept and monoclonal-antibody therapy in the main analysis and in the sensitivity analyses (Figure 3), even when we separately used the different estimates from independent sources, which gave very consistent adjusted incidence rates and SIRs (supplementary Figure 1).

DISCUSSION

This 3-year study is the first national prospective study recording all cases of lymphoma in patients receiving anti-TNF agents, whatever the underlying disease. This study allowed us to collect enough cases to differentiate between lymphoma risk by use of anti-TNF agent. We found higher incidence of lymphoma with use of the two monoclonal-antibody agents (adalimumab and infliximab) than with the soluble-receptor agent (etanercept).

Three cohorts of RA patients have been used to compare treatment with anti-TNF agents and with classical disease-modifying anti-rheumatic drugs in terms of risk of lymphoma;[7 9-11] and did not found an increased risk with anti-TNF agents (relative risk of 1.0 [0.6-1.8][7], 1.35 [0.82-2.11][9] and 1.11 [0.51-2.37][10]) These studies failed to demonstrate a difference between the treatments in risk of lymphoma due to insufficient power. Although the design of the RATIO study has some limitations, it is probably the only way (or at least the most powerful way) to investigate difference in risk with use of anti-TNF agents.

Our study may have some limitations:

The denominator of the incidence rate was estimated only. However, because each firm evaluated the number of patient-years in the period for each anti-TNF agent, the difference in risk between agents we observed cannot be explained by different methodologies used for the different agents. Furthermore, in the sensitivity analyses, the different estimates from independent sources gave very consistent adjusted incidence rates and SIRs (supplementary Figure 1).

Despite the different strategies used to identify all the cases in the whole country, we cannot exclude that some cases were missed. We make the assumption that reporting was equal with each biologic.. Actually, reporting of adverse events could be lower in patients treated sub-cutaneously (SC; i.e. etanercept and adalimumab) outside the hospital. But, in France, the SC-treated patients are mandatory seen by hospital physicians initially and yearly for renewal. Moreover, the lack of AE reporting is a main issue for minor side effects but not for life-threatening side effects, particularly lymphomas that are a major concern for physicians and patients

regarding anti-TNF agents. Furthermore, the patients treated with anti TNF agents that have lymphomas could be notified to RATIO by the anti TNF agent prescribor (rheumatologist, gastro-enterologist, internist or others), by the onco-haematologist, or by the pharmacovigilance regional center. Finally, we found that the risk of lymphoma was similar for adalimumab and infliximab, that share the same mechanism of action (different from the one of etanercep), but adalimumab is a S.C. anti TNF agent and infliximab I. V. anti TNF agent.

Finally, the cumulative activity of the disease, known as a risk factor of lymphoma at least for RA patients,[4] could be different among patients receiving the different anti-TNF agents. Indeed, disease in patients receiving therapy at the beginning of anti-TNF availability (before 2002), was probably more severe, and such patients received exclusively infliximab (the only anti-TNF available in France at that time). However, patients with anti-TNF onset before 2002 did not have a higher risk of lymphoma than others (anti-TNF onset before 2002: OR=1.3 [0.5–3.7]; p=0.60). Furthermore, the comparison between the type of drug used and risk was adjusted on the time from onset of anti-TNF treatment (Table 3). In addition, indirect markers of disease activity (median duration of the inflammatory underlying disease, percentage of patients treated with steroids, frequency of positive rheumatoid factor and anti-CCP in RA patients) were not greater in patients treated with infliximab or adalimumab than in those treated with etanercept (data not shown). Lastly, the impact of a putative difference in duration of exposure and in disease activity depending on year of introduction of the anti-TNF agent probably cannot explain the difference in incidence of lymphoma depending on the type of anti-TNF agent used because we observed exactly the same increased risk of lymphoma for patients receiving infliximab, which was introduced in 1999, and adalimumab, which has been available since 2004, whereas the risk was lower with use of etanercept, available in France from early 2003.

The strengths of this study are that our population of focus was the whole French population receiving anti-TNF therapy, whatever the indication for use, rather than a limited and selected population included in a specific cohort study. Furthermore, all the cases were validated by an expert committee, and the biopsy specimens were centralized, reviewed by the same hematopathologist and tested for EBV.

Even though the overall risk of lymphoma in RA patients treated with anti-TNF therapy does not appear to differ greatly from what is expected in a population of patients with inflammatory diseases,[1-3] the risk differs depending on the anti-TNF drug used (higher risk with monoclonal anti-TNF therapy, adalimumab and infliximab). This difference in risk depending on agent was found in the case-control study and confirmed in the comparison of incidence with the general population, which supports the robustness of this finding. A meta-analysis assessing cancers in randomized controlled trials using monoclonal anti-TNF therapy, adalimumab and infliximab in RA patients revealed 10 cases of lymphoma (4 in randomized phases of trials and 6 in extension phases) in the treated groups (3,493 patient-years) and 0 in placebo groups (1,512 patient-years).[15] The same analysis of randomized controlled trials with etanercept of 2,484 patient-years with etanercept treatment and 1,072 patient-years with control therapy revealed 2 cases of lymphoma with etanercept therapy and none with control therapy.[16] Finally, cases of hepatosplenic T-cell lymphoma have been described in adolescents and young adults with CD treated with monoclonal antibodies and azathioprine combined (10 cases with infliximab[17] and 3 cases with adalimumab.[18])

The absence of intrinsic increased risk of lymphoma in SpA patients makes this population an ideal model for assessing the anti-TNF-related risk of lymphoma. [19] In our study, no significant increase in risk of lymphoma was observed in patients receiving anti-TNF therapy for SpA. However, no definitive conclusion may be drawn from our data based on very few cases of lymphoma in SpA patients. Some of the cases we observed reinforce the likelihood of a causal role of anti-TNF therapy in risk of lymphoma. In one patient with AS who never received other immunosuppressors, including MTX, EBV-associated Hodgkin's-like lymphoma developed after treatment with infliximab. We observed 3 cases of EBV-induced lymphoproliferation: 2 cases of EBV-associated Hodgkin's-like lymphomas with infliximab treatment (one with RA, one with AS described above) and 1 case of EBV-associated B-cell NHL in a patient with RA treated with adalimumab. These 3 cases demonstrate that lymphomas similar to post-transplant lymphoproliferative disease may occur, even rarely, with anti-TNF treatment. In the literature, one case of EBV-associated lymphoproliferation in a patient with RA treated with etanercept regressed after withdrawal of the drug. [20]

Another case of hypopharynx MALT lymphoma not associated with EBV regressed spontaneously after withdrawal of infliximab. [21]

The pathophysiological mechanism inducing a higher risk of lymphoma in patients receiving anti-TNF therapy remains unclear. A direct action of TNF or anti-TNF on B cells was hypothesized, but no increase in survival or apoptosis with TNF or infliximab treatment was found. [23]

Actually, in inflammatory diseases and especially RA, the 3 anti-TNF agents may have opposite effects: a beneficial effect due to the decrease in activity of the disease and a deleterious effect due to immunomodulatory activity, which may concern EBV-associated lymphoma but also more classical lymphoma. The mechanism of action of this deleterious effect is still unknown but could be related to T-cell control of viruses such as EBV or of other mechanisms of lymphomagenesis. This T-cell control may require integrity of membrane TNF, which is upregulated in activated T cells. Some studies suggest a higher efficacy of anti-TNF monoclonal-antibody treatment than TNF soluble receptor therapy for inhibiting membrane TNF signaling,[22] which could lead to a decreased immune surveillance of different mechanisms of lymphomagenesis.

In conclusion, some lymphomas associated with immunosuppression may occur in such patients. The incidence of lymphoma is higher with monoclonal-antibody agents than with the soluble receptor. This may be due to a difference of targeting membrane TNF, leading also to difference of effectiveness in some diseases such as Crohn's disease or granulomatous diseases.

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The authors' contributions were as follows:
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- Analysis and interpretation of data: Mariette, Tubach, Ravaud
- Drafting the article and revising it critically for important intellectual content: Mariette, Tubach, Ravaud
- Final approval of the version to be published: Mariette, Tubach, Bagheri, Bardet, Berthelot, Gaudin, Heresbach, Martin, Schaeverbeke, Salmon, Lemann, Hermine, Raphael, Ravaud

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References

1. Zintzaras E, Voulgarelis M, Moutsopoulos HM. The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch Intern Med* 2005;165(20):2337-44.
2. Smedby KE, Hjalgrim H, Askling J, Chang ET, Gregersen H, Porwit-MacDonald A, et al. Autoimmune and chronic inflammatory disorders and risk of non-Hodgkin lymphoma by subtype. *Journal of the National Cancer Institute* 2006;98(1):51-60.
3. Landgren O, Engels EA, Pfeiffer RM, Gridley G, Mellemkjaer L, Olsen JH, et al. Autoimmunity and susceptibility to Hodgkin lymphoma: a population-based case-control study in Scandinavia. *Journal of the National Cancer Institute* 2006;98(18):1321-30.
4. Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 2006;54(3):692-701.
5. Kandiel A, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. *Gut* 2005;54(8):1121-5.
6. Kamel OW, van de Rijn M, Weiss LM, Del Zoppo GJ, Hench PK, Robbins BA, et al. Brief report: reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *N Engl J Med* 1993;328(18):1317-21.
7. Wolfe F, Michaud K. The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. *Arthritis Rheum* 2007;56(5):1433-9.
8. Mariette X, Cazals-Hatem D, Warszawki J, Liote F, Balandraud N, Sibilia J. Lymphomas in rheumatoid arthritis patients treated with methotrexate: a 3-year prospective study in France. *Blood* 2002;99(11):3909-15.
9. Askling J, Baecklund E, Granath F, Geborek P, Fored M, Backlin C, et al. Anti-TNF therapy in RA and risk of malignant lymphomas Relative risks and time-trends in the Swedish Biologics Register. *Ann Rheum Dis* 2009;68(5):Epub 2008 May 8.
10. Setoguchi S, Solomon DH, Weinblatt ME, Katz JN, Avorn J, Glynn RJ, et al. Tumor necrosis factor alpha antagonist use and cancer in patients with rheumatoid arthritis. *Arthritis Rheum* 2006;54(9):2757-64.
11. Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum* 2004;50(6):1740-51.
12. Tubach F, Salmon D, Ravaud P, Allanore Y, Goupille P, Bréban M, et al. Risk of tuberculosis higher with monoclonal-antibody than with soluble-receptor anti-TNF therapy in the 3-year prospective French RATIO registry. *Arthritis Rheum* 2009;In press.
13. Tubach F, Salmon-Ceron D, Ravaud P, Mariette X. The RATIO observatory: French registry of opportunistic infections, severe bacterial infections, and lymphomas complicating anti-TnFalpha therapy. *Joint Bone Spine* 2005;25:25.
14. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007;4(10):e296.
15. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *Jama* 2006;295(19):2275-85.
16. Bongartz T, Warren F, Mines D, Matteson E, Abrams K, Sutton A. Etanercept therapy in rheumatoid arthritis and the risk of malignancies. *Ann Rheum Dis* 2008;67((suppl II)):OP-0012.
17. Mackey AC, Green L, Liang LC, Dinndorf P, Avigan M. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. *Journal of pediatric gastroenterology and nutrition* 2007;44(2):265-7.
18. Agency EM. Committee for medicinal products for human use, 2008.
19. Park SH, Kim CG, Kim JY, Choe JY. Spontaneous regression of EBV-associated diffuse lymphoproliferative disease in a patient with rheumatoid arthritis after discontinuation of etanercept treatment. *Rheumatology international* 2008;28(5):475-7.
20. Thonhofer R, Gaugg M, Kriessmayr M, Neumann HJ, Erlacher L. Spontaneous remission of marginal zone B cell lymphoma in a patient with seropositive rheumatoid arthritis after discontinuation of infliximab-methotrexate treatment. *Ann Rheum Dis* 2005;64(7):1098-9.

21. Baran-Marszak F, Laguillier C, Youlyouz I, Feuillard J, Mariette X, Fagard R, et al. Effect of tumor necrosis factor alpha and infliximab on apoptosis of B lymphocytes infected or not with Epstein-Barr virus. *Cytokine* 2006;33(6):337-45.
22. Scallon B, Cai A, Solowski N, Rosenberg A, Song XY, Shealy D, et al. Binding and functional comparisons of two types of tumor necrosis factor antagonists. *The Journal of pharmacology and experimental therapeutics* 2002;301(2):418-26.

Table 1. Characteristics of the 38 lymphoma cases

	All lymphoma (n=38)	**Lymphoma in RA patients** (n=27)
Age (years)	61.3 ± 12.3 (63.5)	63.4 ± 11.8 (64.0)
Sex (female)	17 (44.7%)	15 (55.6%)
Underlying inflammatory disease		
RA	27 (71.0%)	27
Ankylosing spondylitis	4 (10.5%)	
Psoriatic arthritis	3 (7.9%)	
Crohn's disease	3 (7.9%)	
Primary Sjögren's syndrome	1 (2.6%)	
Duration of the underlying inflammatory disease before the first symptoms of lymphoma (years)	11.0 ± 8.6 (8.9)	11.3 ± 9.0 (8.2)
Activity of the underlying inflammatory disease at the time of diagnosis of lymphoma		
Null	9 (26.5%)	7 (29.2%)
Fair	8 (23.5%)	5 (20.8%)
Moderate	7 (20.6%)	5 (20.8%)
High	10 (29.4%)	7 (29.2%)
Lymphoma histological subtype		
Non-Hodgkin's lymphoma	**31 (81.6%)**	**22 (81.5%)**
- B-cell lymphoma	26 (83.9%)	20 (74.1%)
Diffuse large B-cell	14	11
Follicular B-cell	4	3
Marginal zone B-cell	2	1
Lymphocytic	2	1
Others	4	4
- T-cell lymphoma	5 (16.1%)	2 (7.4%)
Pleiomorphic T-cell	3	2
Sezary T-cell	1	0
Lymphoblastic T-cell	1	0
Hodgkin's and Hodgkin's-like lymphoma	7 (18.4%)	5 (18.5%)
- Hodgkin-like	2	1
- Scleronodular	2	1

- mixed cellularity	3	3
Anti-TNF treatment		
Number of anti-TNF agents received		
1	31 (81.6%)	20 (74.1%)
2	6 (15.8%)	6 (22.2%)
3	1 (2.6%)	1 (3.7%)
First anti-TNF agent received		
Adalimumab	8 (21.0%)	8 (29.6%)
Etanercept	11 (29.0%)	8 (29.6%)
Infliximab	19 (50.0%)	11 (40.7%)
Last anti-TNF agent received		
Adalimumab	12 (31.6%)	12 (44.4%)
Etanercept	7 (18.4%)	4 (14.8%)
Infliximab	19 (50.0%)	11 (40.7%)
Ever used Adalimumab	12 (31.6%)	12 (44.4%)
Ever used Etanercept	13 (34.2%)	10 (37.0%)
Ever used Infliximab	21 (55.3%)	13 (48.1%)
Ever used Infliximab or adalimumab	32 (84.2%)	24 (88.9%)
Time since first anti-TNF treatment began[§] (months)	27.0 ± 16.7 (23.6)	29.2 ± 17.2 (25.6)
Time since last anti-TNF treatment began[§] (months)	23.7 ± 16.0 (22.7)	24.6 ± 16.6 (22.5)
DMARD use during the last 5 years		
Methotrexate	27 (71.1%)	23 (85.2%)
Azathioprine	3 (7.9%)	0 (0.0%)
Leflunomide	7 (18.4%)	7 (25.9%)

RA: rheumatoid arthritis; DMARD: disease-modifying anti-rheumatic drug
Continuous variables are mean ± SD (median)
Categorized variables are numbners (%)
§ Time from onset of first/last anti-TNF treatment to first symptoms of lymphoma

Table 2: Risk factors of lymphoma for patients receiving anti-TNF agents (univariate analysis: main analysis and analysis restricted to rheumatoid arthritis [RA] patients)

	All lymphoma (37 cases and 74 controls)				**Lymphoma in RA patients** (27 cases and 54 controls)			
	Cases (n=37)	**Controls (n=74)**	**OR [95% CI]**	**p value**	**Cases (n=27)**	**Controls (n=54)**	**OR [95% CI]**	**p value**
Duration of the underlying inflammatory disease (years)	11.07 (8.75)	16.5 ± 12.4	0.95 [0.91 – 1.00]	0.047	11.26 (8.97)	17.46 (12.12)	0.94 [0.88 – 1.00]	0.06
Activity of the underlying inflammatory disease at the time of diagnosis of lymphoma								
Null, fair, or moderate	23 (69.7%)	42 (85.7%)	1	0.22	17 (70.8%)	33 (89.2%)	1	0.15
High	10 (30.3%)	7 (14.3%)	2.04 [0.65 – 6.44]		7 (29.2%)	4 (10.8%)	2.84 [0.69 – 11.67]	
Number of anti-TNF agents received								
1	30 (81.1%)	56 (75.7%)	1	0.51	20 (74.1%)	40 (74.1%)	1	1.00
2 or 3	7 (18.9%)	18 (24.3%)	0.71 [0.25 – 1.97]		7 (25.9%)	14 (25.9%)	1.00 [0.33 – 3.04]	
First anti-TNF agent received								
Etanercept	11 (29.7%)	30 (40.5%)	1					
Adalimumab	8 (21.6%)	9 (12.2%)	2.64 [0.74 – 9.41]	0.33				
Infliximab	18 (48.6%)	35 (47.3%)	1.38 [0.55 – 3.45]					
First anti-TNF agent received								
Etanercept	11 (29.7%)	30 (40.5%)	1		8 (29.6%)	26 (48.1%)	1	
Adalimumab or infliximab	26 (70.3%)	44 (59.5%)	1.61 [0.69 – 3.78]	0.27	19 (70.4%)	28 (51.9%)	2.05 [0.79 – 5.27]	0.14
Last anti-TNF agent received								
Etanercept	7 (18.9%)	33 (44.6%)	1					
Adalimumab	12 (32.4%)	13 (17.6%)	4.52 [1.39 – 14.71]	0.03				
Infliximab	18 (48.6%)	28 (37.8%)	3.00 [1.10 – 8.15]					
Last anti-TNF agent received								
Etanercept	7 (18.9%)	33 (44.6%)	1		4 (14.8%)	29 (53.7%)	1	
Adalimumab or infliximab	30 (81.1%)	41 (55.4%)	3.40 [1.31 – 8.80]	0.012	23 (85.2%)	25 (46.3%)	6.68 [1.90 – 23.54]	0.003
Time from onset of first anti-								

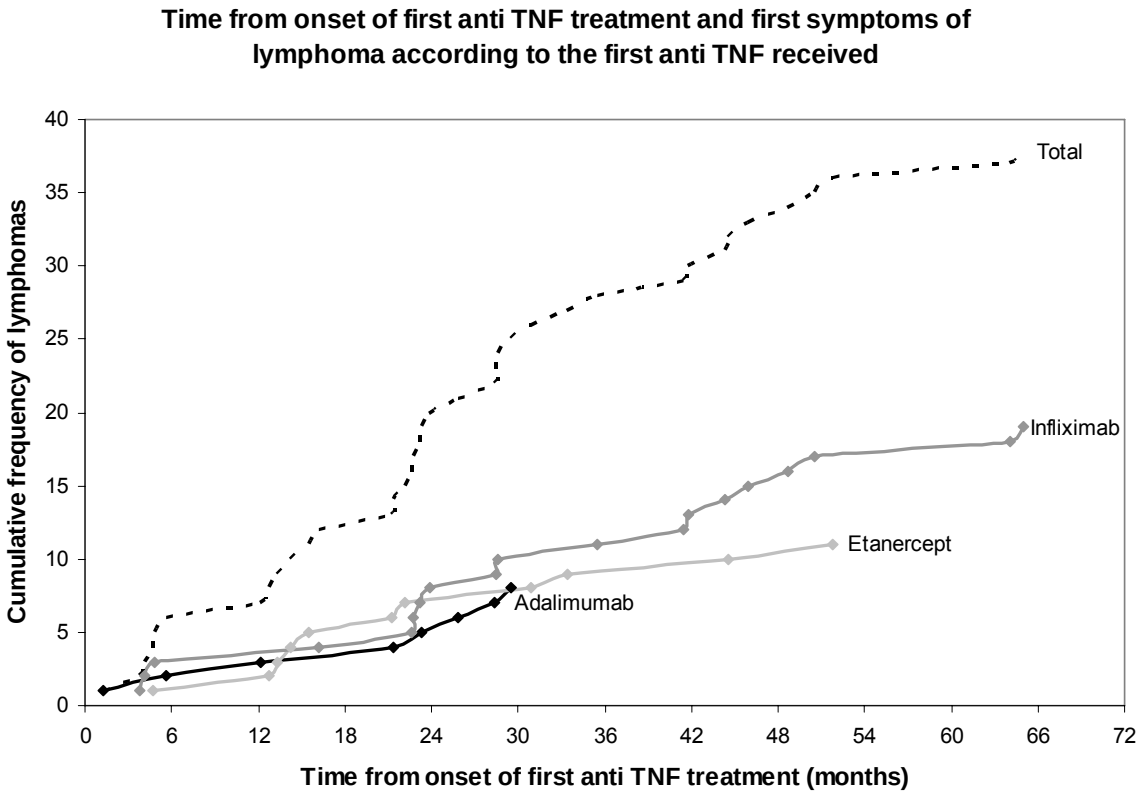
TNF treatment[¥]								
< 2 years	20 (54.1%)	24 (32.4%)	2.71 [1.09 – 6.73]	0.03	14 (51.9%)	38 (70.4%)	2.46 [0.83 – 7.26]	0.10
≥ 2 years	17 (45.9%)	50 (67.6%)	1 (Ref)		13 (48.1%)	16 (29.6%)	1 (Ref)	
Time from onset of last anti-TNF treatment[¥]								
< 2 years	23 (62.2%)	31 (41.9%)	2.37 [1.00 – 5.60]	0.05	16 (59.3%)	23 (42.6%)	2.05 [0.75 – 5.61]	0.16
≥ 2 years	14 (37.8%)	43 (58.1%)	1 (Ref)		11 (40.7%)	31 (57.4%)	1 (Ref)	
Year of onset of first anti-TNF agent use before 2002								
No	29 (78.4%)	61 (82.4%)	1	0.60	21 (77.8%)	42 (77.8%)	1	1.00
Yes	8 (21.6%)	13 (17.6%)	1.32 [0.47 – 3.69]		6 (22.2%)	12 (22.2%)	1.00 [0.32 – 3.17]	
Etanercept use								
Never	24 (64.9%)	35 (47.3%)	1	0.08	17 (63.0%)	20 (37.0%)	1	0.04
Ever	13 (35.1%)	39 (52.7%)	0.45 [0.19 – 1.09]		10 (37.0%)	34 (63.0%)	0.35 [0.13 – 0.94]	
Infliximab or adalimumab use								
Never	6 (16.2%)	24 (32.4%)	1		3 (11.1%)	21 (38.9%)	1	
Ever	31 (83.8%)	50 (67.6%)	2.36 [0.89 – 6.27]	0.08	24 (88.9%)	33 (61.1%)	4.59 [1.27 – 16.56]	0.02
Methotrexate[$]								
No	11 (29.7%)	18 (24.3%)	1	0.52	4 (14.8%)	11 (20.4%)	1	0.54
Yes	26 (70.3%)	56 (75.7%)	0.73 [0.29 – 1.87]		23 (85.2%)	43 (79.6%)	1.50 [0.41 – 5.54]	
Azathioprine[$]								
No	34 (91.9%)	66 (89.2%)	1	0.49	27 (100.0%)	51 (94.4%)	1	
Yes	3 (8.1%)	8 (10.8%)	0.43 [0.04 – 4.61]		0 (0.0%)	3 (5.6%)	-	-
Leflunomide[$]								
No	30 (81.1%)	58 (78.4%)	1	0.73	20 (74.1%)	38 (70.4%)	1	0.73
Yes	7 (18.9%)	16 (21.6%)	0.83 [0.30 – 2.34]		7 (25.9%)	16 (29.6%)	0.83 [0.30 – 2.34]	

Continuous variables are mean (SD)
Categorized variables are numbers (%)
OR: odds ratio
¥ Time from onset of last anti-TNF treatment and first symptoms of lymphoma for cases, time from onset of last anti-TNF treatment and last news for controls
$ Treatment during the last 5 years

Table 3: Risk factors of lymphoma for patients receiving anti-TNF agents (multivariate analysis: main analysis and analysis restricted to rheumatoid arthritis [RA] patients)

	All lymphoma (37 cases and 74 controls)	
	OR [95% CI]	**p value**
Last anti-TNF agent received		
Etanercept	1	
Adalimumab	4.73 [1.27 – 17.65]	0.02
Infliximab	4.12 [1.36 – 12.49]	0.01
Time from onset of first anti-TNF treatment[¥]		
< 2 years	3.30 [1.17 – 9.30]	0.02
≥ 2 years	1 (Ref)	
	Lymphoma in RA patients (27 cases and 54 controls)	
Last anti-TNF agent received		
Etanercept	1	0.003
Adalimumab or infliximab	6.68 [1.90 – 23.54]	

Figure 1: Time from onset of first and last anti-TNF treatment and first symptoms of lymphoma (months)



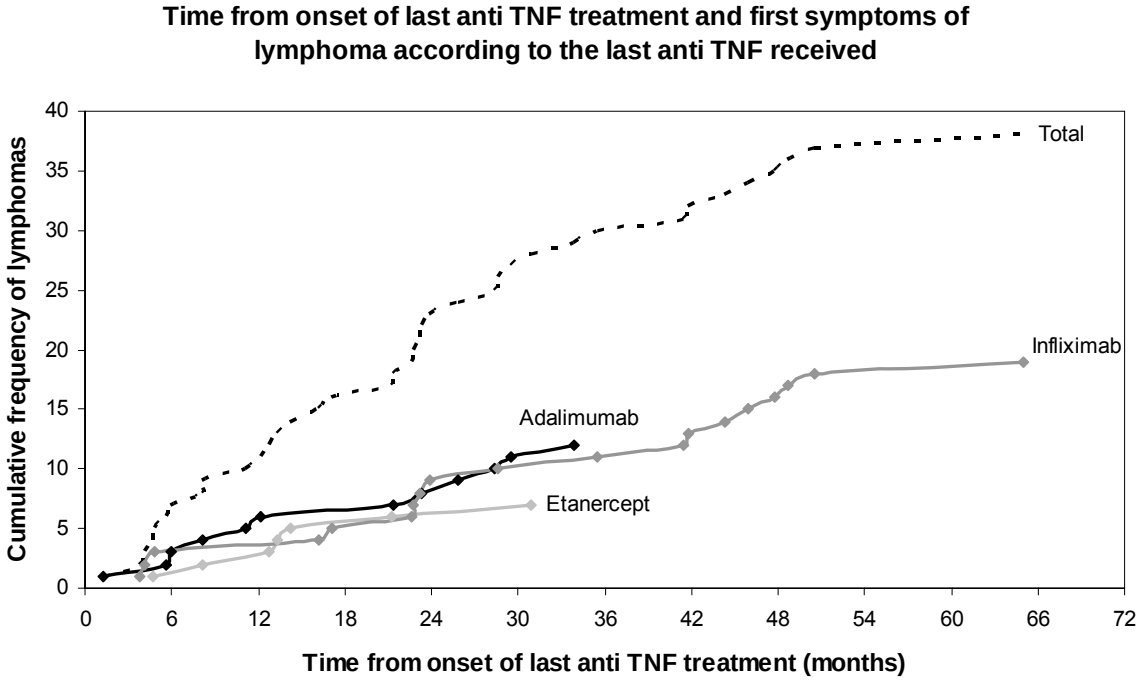
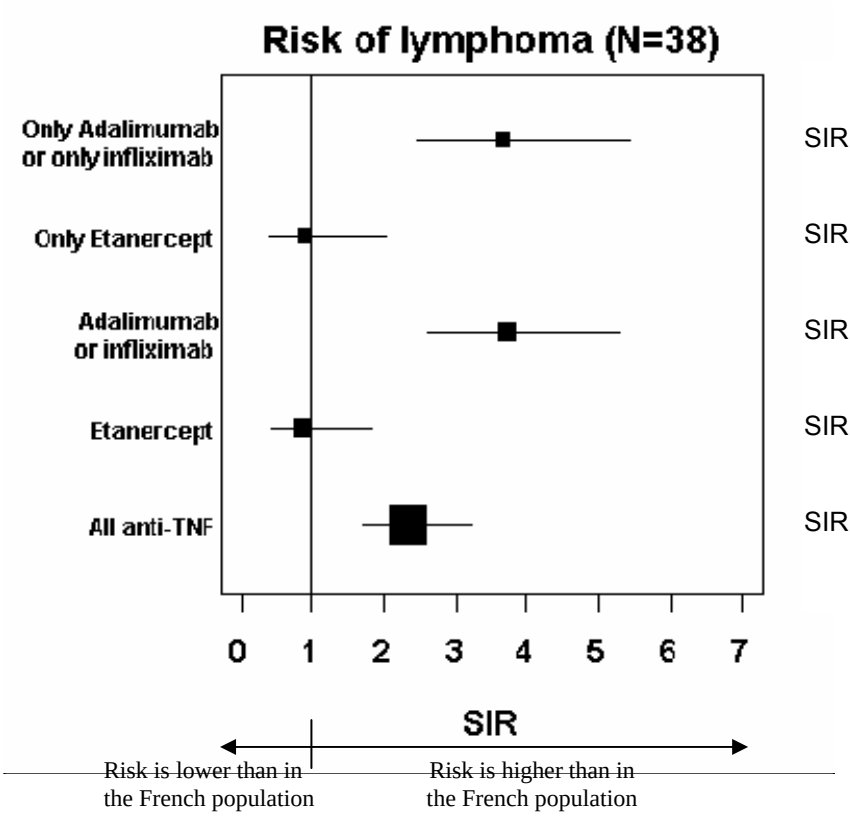
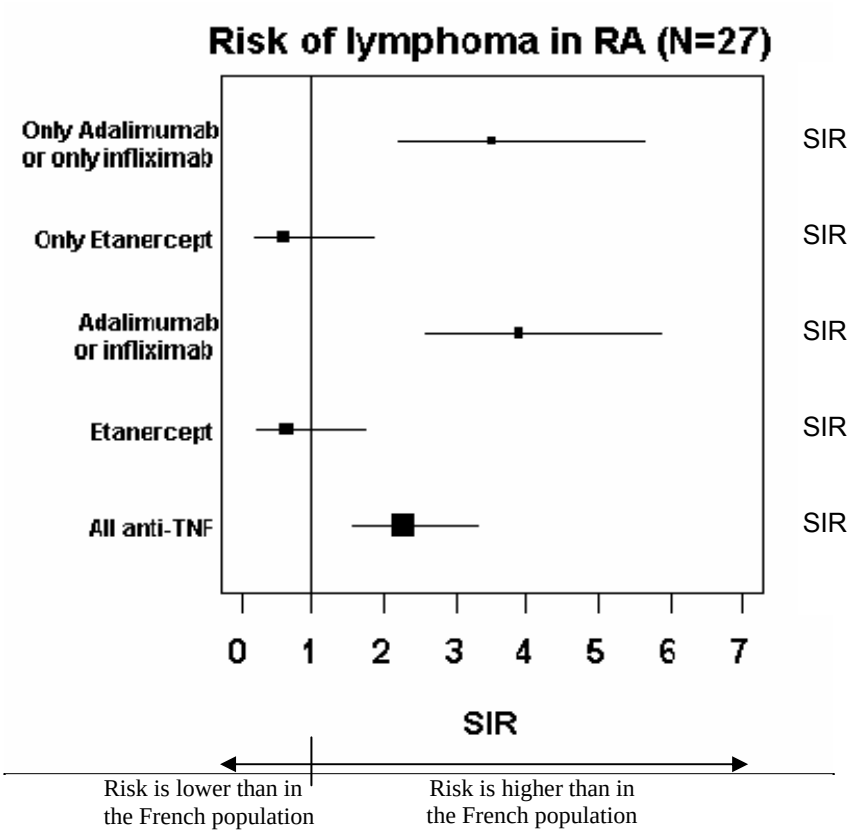


Figure 2: Estimation of the standardized incidence ratio (SIR) for risk of lymphoma according to underlying disease, and the histological subtype of lymphoma

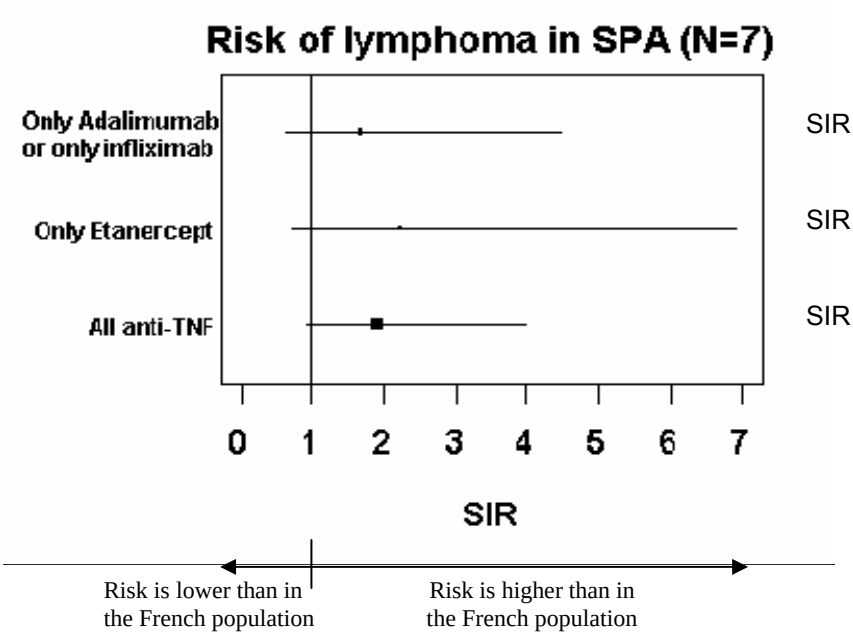


SIR = 3.7 [2.5 – 5.4]	p<0.0001	n=25*
SIR = 0.9 [0.4 – 2.0]	p=0.82	n=6
SIR = 3.7 [2.6 – 5.3]	p<0.0001	n=31
SIR = 0.9 [0.4 – 1.8]	p=0.72	n=7
SIR = 2.4 [1.7 – 3.2]	p<0.0001	n=38

* Only INF: n=17, SIR = 3.9 [2.1 – 6.2]
Only ADA: n=8, SIR = 3.3 [1.7 – 6.6]



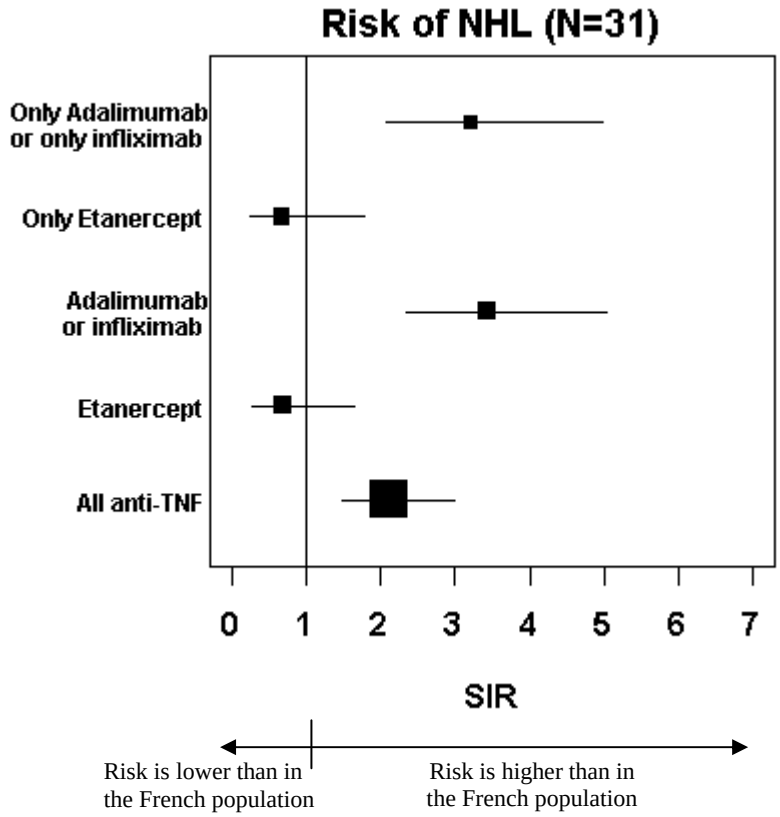
SIR = 3.5 [2.2 – 5.7]	p<0.0001	n=17
SIR = 0.6 [0.2 – 1.9]	p=0.37	n=3
SIR = 3.9 [2.6 – 5.9]	p<0.0001	n=23
SIR = 0.7 [0.2 – 1.7]	p=0.39	n=4
SIR = 2.3 [1.6 – 3.3]	p<0.0001	n=27



SIR = 1.7 [0.6 – 4.5] p=0.30 n=4

SIR = 2.2 [0.7 – 6.9] p = 0.16 n=3

SIR = 1.9 [0.9 – 4.0] p=0.09 n=7



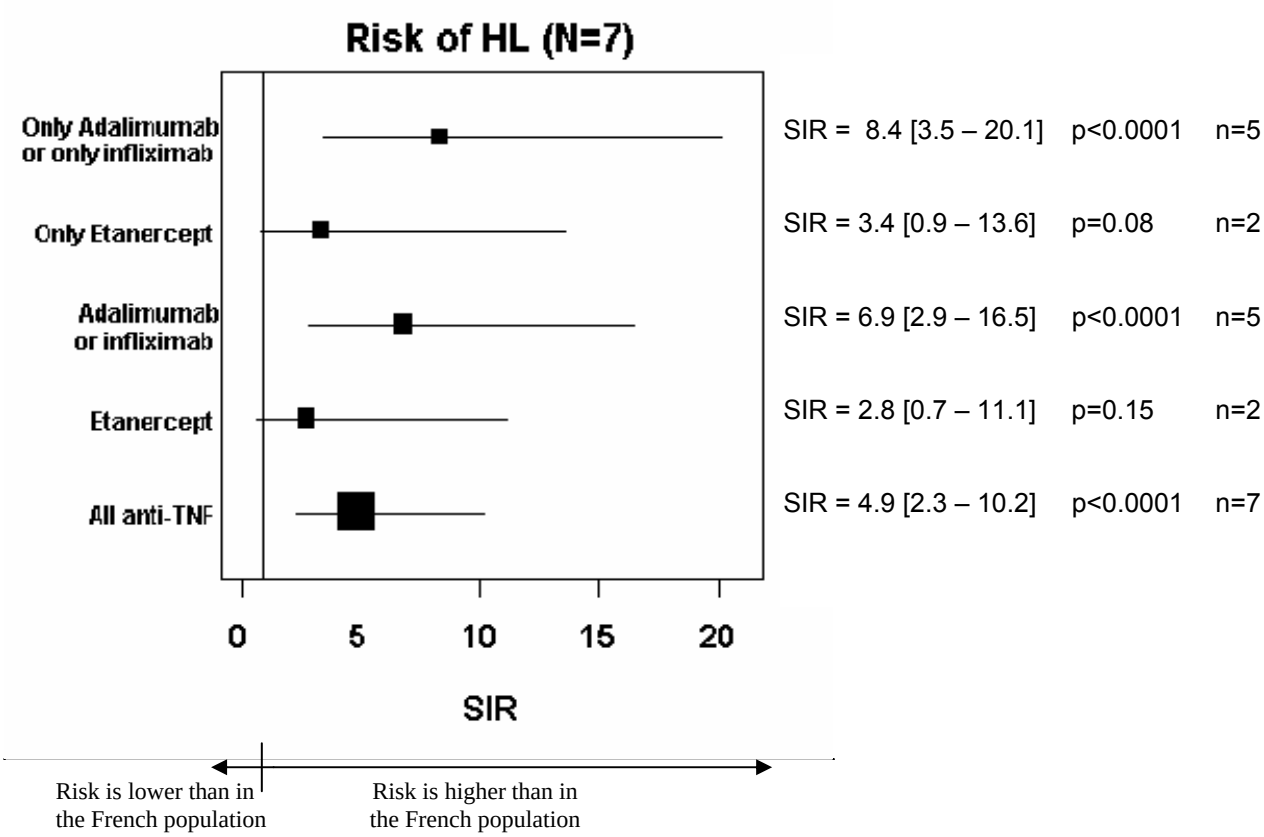
SIR = 3.2 [2.1 – 4.9] p=<0.0001 n=20

SIR = 0.7 [0.3 – 1.8] p=0.42 n=4

SIR = 3.4 [2.3 – 5.0] p<0.0001 n=26

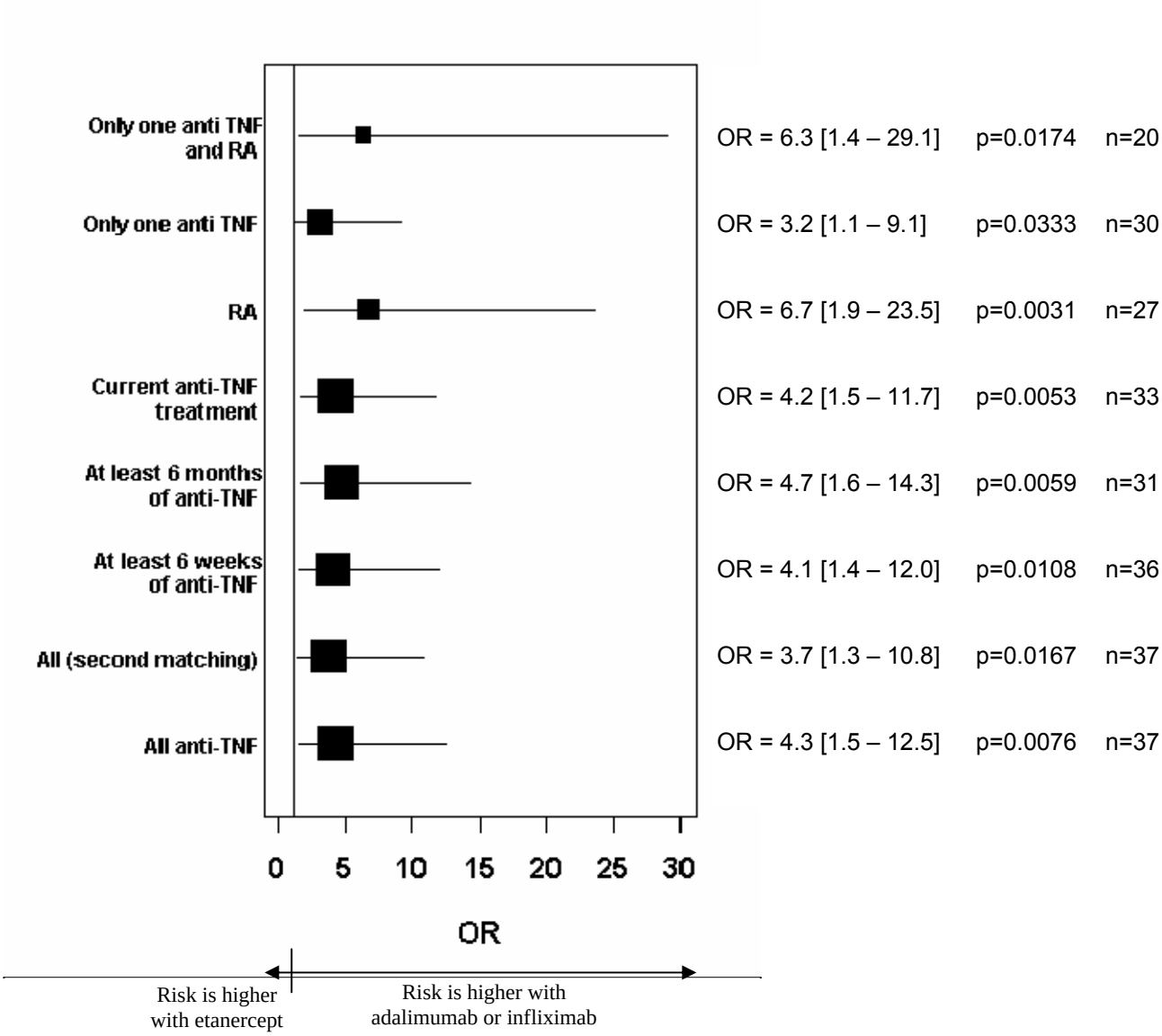
SIR = 0.7 [0.3 – 1.7] p=0.40 n=5

SIR = 2.1 [1.5 – 3.0] p<0.0001 n=31



n is the number of cases involved in the calculation (numerator of the incidence rate)
the plot size relates to the number of patients treated involved in the calculation (denominator of the incidence rate)

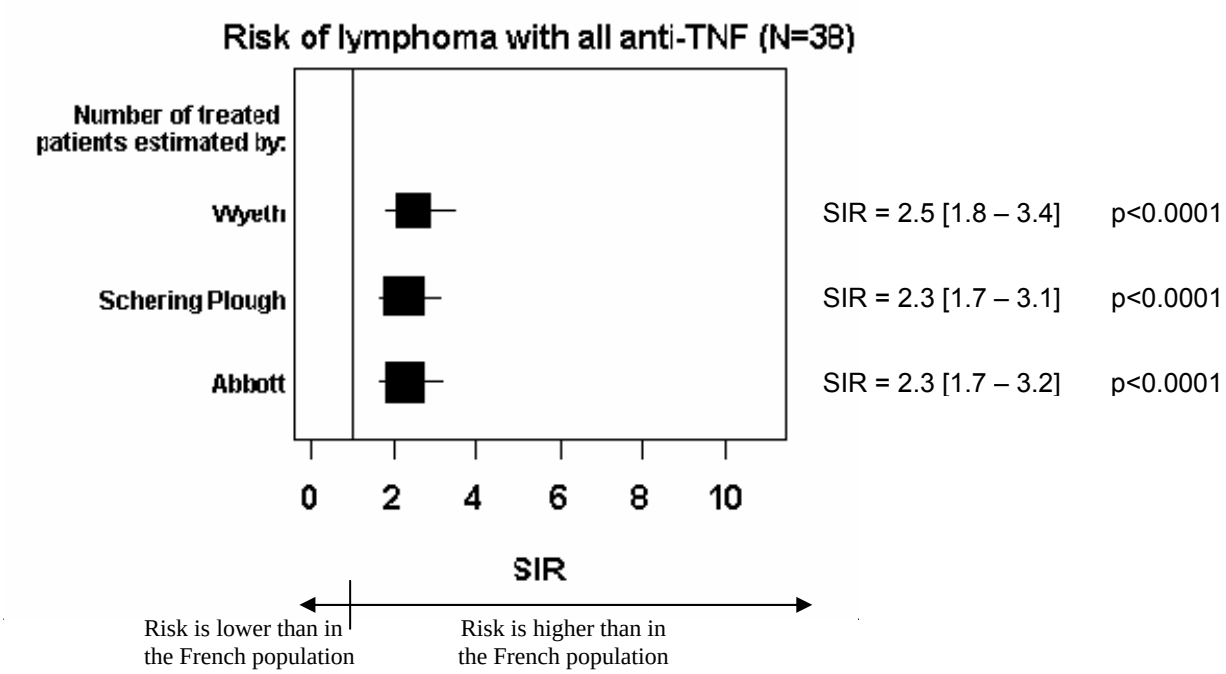
Figure 3 : Sensitivity analysis of the results of the case-control analysis: odds ratios (ORs) for the risk of being treated with adalimumab or infliximab rather than with etanercept in multivariate analysis.

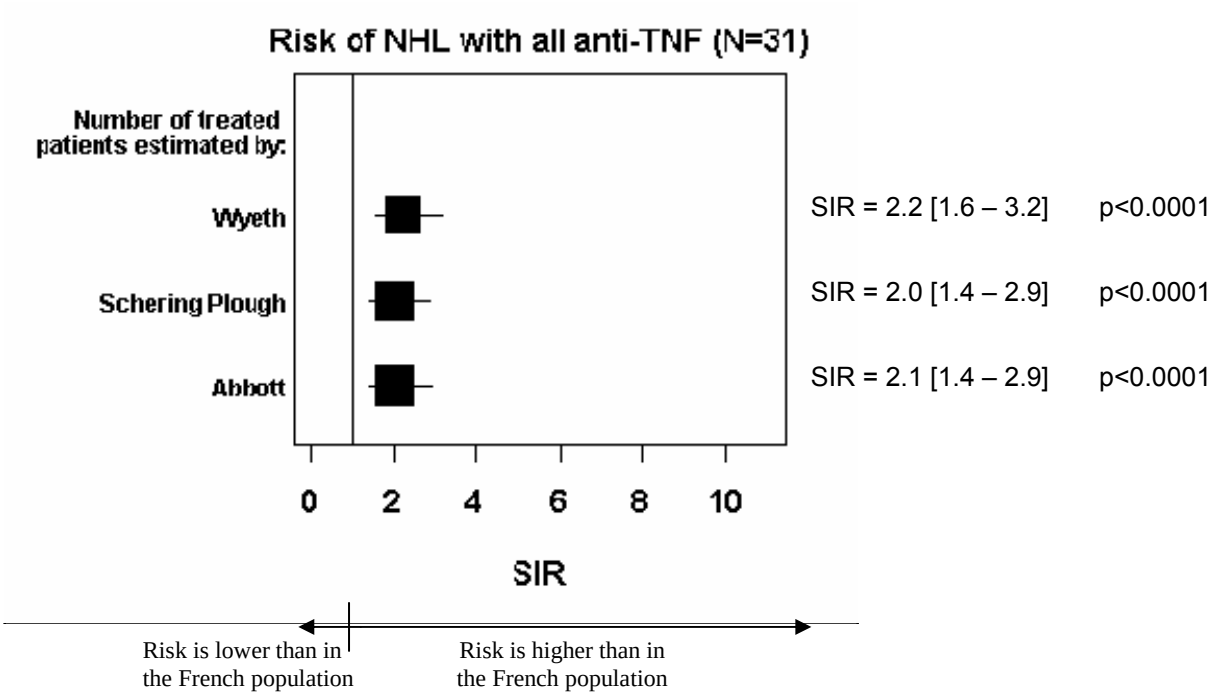


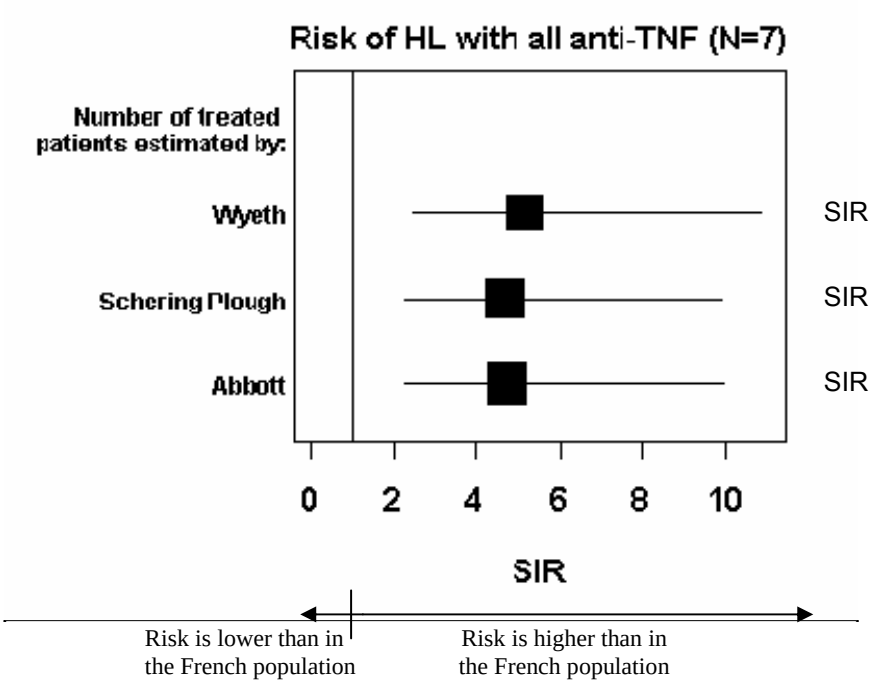
n is the number of cases involved in the calculation
the plot size relates to the number of patients treated involved in the calculation

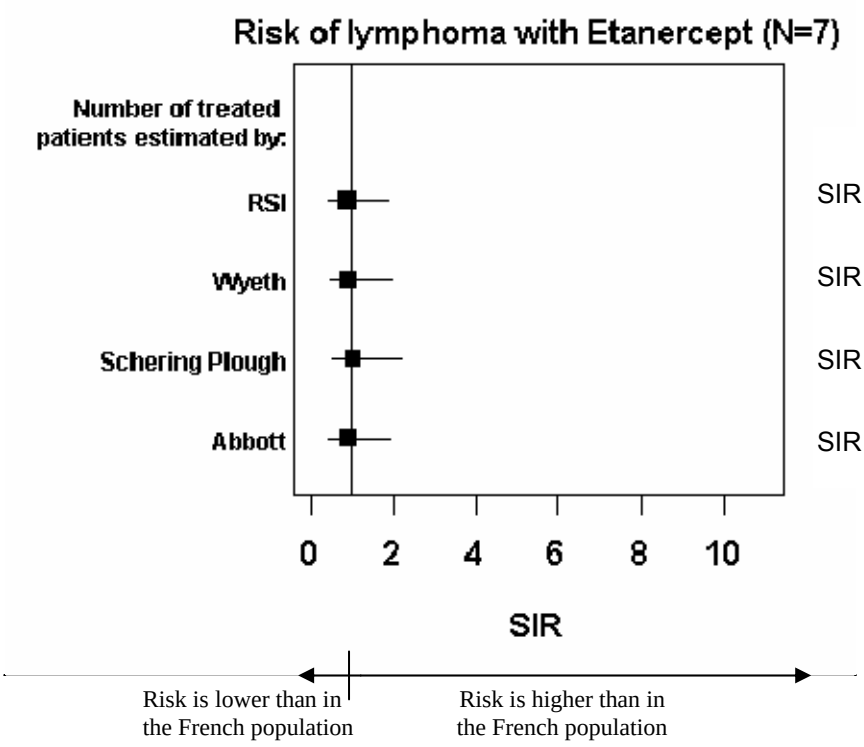
Supplementary figure

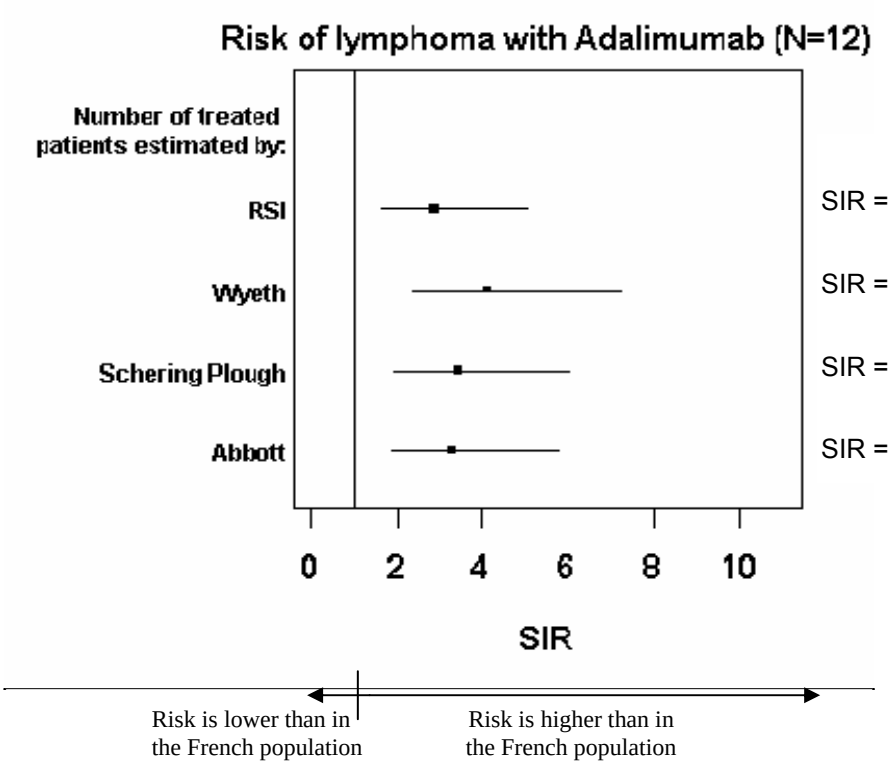
Figure 1: Sensitivity analysis of the estimation of the standardized incidence ratio (SIR) for the risk of lymphoma when using the denominator estimated by each pharmaceutical company and by the RSI (French Sickness Insurance Fund for self-employed workers – providing claims data for etanercept and adalimumab).

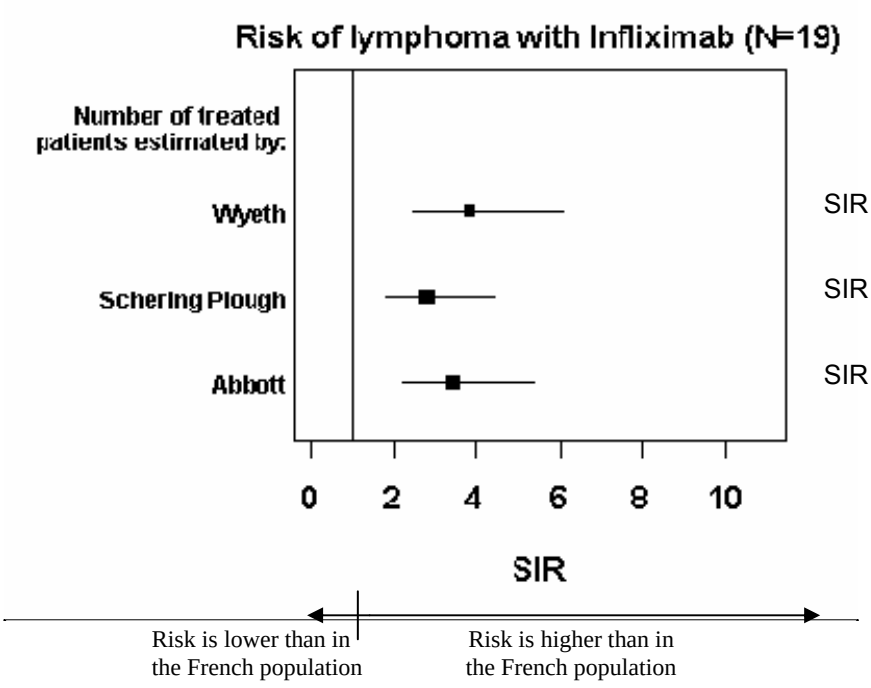





Risk of HL with all anti-TNF (N=7)
Number of treated patients estimated by:
Wyeth
Schering Plough
Abbott
SIR = 5.2 [2.5 – 10.8] p<0.0001
SIR = 4.7 [2.2 – 9.9] p<0.0001
SIR = 4.7 [2.3 – 9.9] p<0.0001
0 2 4 6 8 10
SIR
Risk is lower than in the French population
Risk is higher than in the French population


Risk of lymphoma with Etanercept (N=7)
Number of treated patients estimated by:
RSI
Wyeth
Schering Plough
Abbott
SIR = 0.9 [0.4 – 1.9] p=0.77
SIR = 0.9 [0.4 – 1.9] p=0.84
SIR = 1.0 [0.5 – 2.2] p=0.92
SIR = 0.9 [0.4 – 1.9] p=0.80
0 2 4 6 8 10
SIR
Risk is lower than in the French population
Risk is higher than in the French population





n is the number of cases involved in the calculation (numerator of the incidence rate)
the plot size relates to the number of patients treated involved in the calculation (denominator of the incidence rate)