SECTION 3. DISCUSSION AND CONCLUSION

1. Discussion

Main results

- Largest study of autoimmune disorders in young females, with clinical recruitment and confirmation of diagnosis
- None of the associations showed a statistically significant increase of risk
- Variable results were observed for the association between individual autoimmune disorders and vaccination by HPV
- A statistically significant lower odds ratio was observed for multiple sclerosis and autoimmune thyroiditis and HPV vaccination, which is discussed further below.
- Overall, a statistically significant lower odds ratio was observed for all studied AID and HPV vaccination, which is discussed further below.
- Results for Cervarix® alone did not seem to differ from those observed for all HPV vaccination; however the exposure of cases was small (only 2 AID cases exposed).

Cases

- Recruitment targets were achieved globally (705 vs an objective of 624 for the 2 periods).
- Recruitments were variable according to the disease:
    - Targets achieved for CD/MS, T1D, AIT and ITP
    - Targets were not achieved for CTD and GBS
- Diagnostic certainty was highly specific with only 0.9% rejected cases.
    - Validation of cases showed that specialists ensured a high clinical quality of cases all over the study recruitment.
    - Possible cases are likely to be confirmed with follow-up as they are recruited on first symptoms and classified as AID based on specialists' opinion.

Referents & Controls

- Recruitment targets of referents and controls were achieved globally, PGRx referents providing a sufficient number of matched controls, above targets.
- Appropriate matching occurred for all individual disorders and for all AID combined, with an average of matched controls per cases close to target (4 expected).
- Cases and control series were very similar on factors of interest except for variable potentially associated with risk (eg: history of autoimmune disorders, exposure to vaccines).

Risk factors

- A personal or familial history of autoimmune disorder was a strong risk factor for all AID combined (OR= 1.86 [95%CI: 1.33 - 2.60], however it showed variations between individual AID.
- Southern origin (both parents) was associated with a higher risk of AID (OR=3.68 [95%CI: 2.76 – 4.89]).
- Smoking and alcohol use were not associated with the risk of AID.

Exposure

- Average exposure of referents is consistent with sales figures globally with a 5% difference; Differences with sales figures may be underreporting by patients, overestimation of actual

use by sales figures due to non adherence to prescription; if true, this difference would be artificially inflating the estimation of the risk.
- Curves and dynamic of exposure by birth cohort were close to expectations.
- The exposure of referents to Cervarix® was low over the whole period (1.5%), although increasing proportionally recently (3.1% in the phase II of the study).
- Propensity of use of an HPV vaccine was not significantly different in patients with a personal or familial history of autoimmune disorder than in patients without such an history or not knowing about this history (OR= 1.18, – 95%CI: 0.83-1.68).
- Propensity of use an HPV vaccine was significantly lower in persons with a geographical origin from Southern countries (OR= 0.53 [95%CI: 0.36 - 0.76]).
- Use of other vaccines was significantly associated with the propensity to use HPV vaccines (OR= 1.32 [95%CI: 1.09 - 1.59]).
- The use of an oral contraceptive was associated with the probability to be exposed to HPV vaccination (OR= 1.73 [95%CI: 1.39 - 2.14])

Confounding

- A higher probability of exposure to HPV vaccine may exist in patients with a higher risk of autoimmune disorder due to personal or familial history of AID.
- Conversely, patients from Southern origin, who show a higher risk of certain AID, may also be less likely to use HPV vaccines.
- Other markers of potential confounding are other vaccination and use of oral contraceptives.
- Analyses stratified by geographical origin and by history of AID did not display significantly different estimates than the multivariate analyses adjusted for all potential confounders
- Remaining, unmeasured, confounding cannot be excluded to explain the lower risk observed for HPV vaccines and certain AID.

2. Conclusion

- No increased risk of autoimmune disorder was observable as associated with HPV vaccination.
- The results observed with the lower used vaccine (Cervarix®) were in accordance with observations in the overall sample.
- The apparent lower risk observed with the occurrence of multiple sclerosis/central demyelination and autoimmune thyroiditis has to be further explored as it could be due to remaining unmeasured confounding, chance or, protection conferred by the vaccination itself.

SECTION APPENDICES AND REFERENCES

Appendix - CNS Demyelination (Group 1)

Table 1. Case definition

Case definitions for the study of central demyelination of the optic nerve

	Clinical presentation		MRI or CSF findings
Definite cases	Optic neuritis	AND	MRI showing T2-weighted hyperintense lesions OR a T1-weighted gadolinium enhancing lesion on the affected optic nerve OR typical CSF findings
Possible cases	Optic neuritis	AND	Encephalic MRI normal (excluding another diagnosis)

Case definitions for the study of central demyelination of the spinal cord or the brain, the brainstem and the cerebellum

	Clinical presentation		MRI or CSF findings
Definite cases	Spinal cord: Myelitis	AND	Spinal or encephalic MRI with T1-weighted gadolinium enhancing lesion or T2-weighted hyperintense lesions OR typical CSF findings
	Brain, brainstem or cerebellum: monofocal or multifocal neurologic signs of progressive evolution	AND	Encephalic MRI as above
Possible cases	Myelitis or monofocal or multifocal neurologic sign	AND	A spinal or encephalic MRI reported as showing hyperintense lesions

Figure 1. Flow chart showing recruitment of central demyelination (CD/MS) cases used in the combined analysis

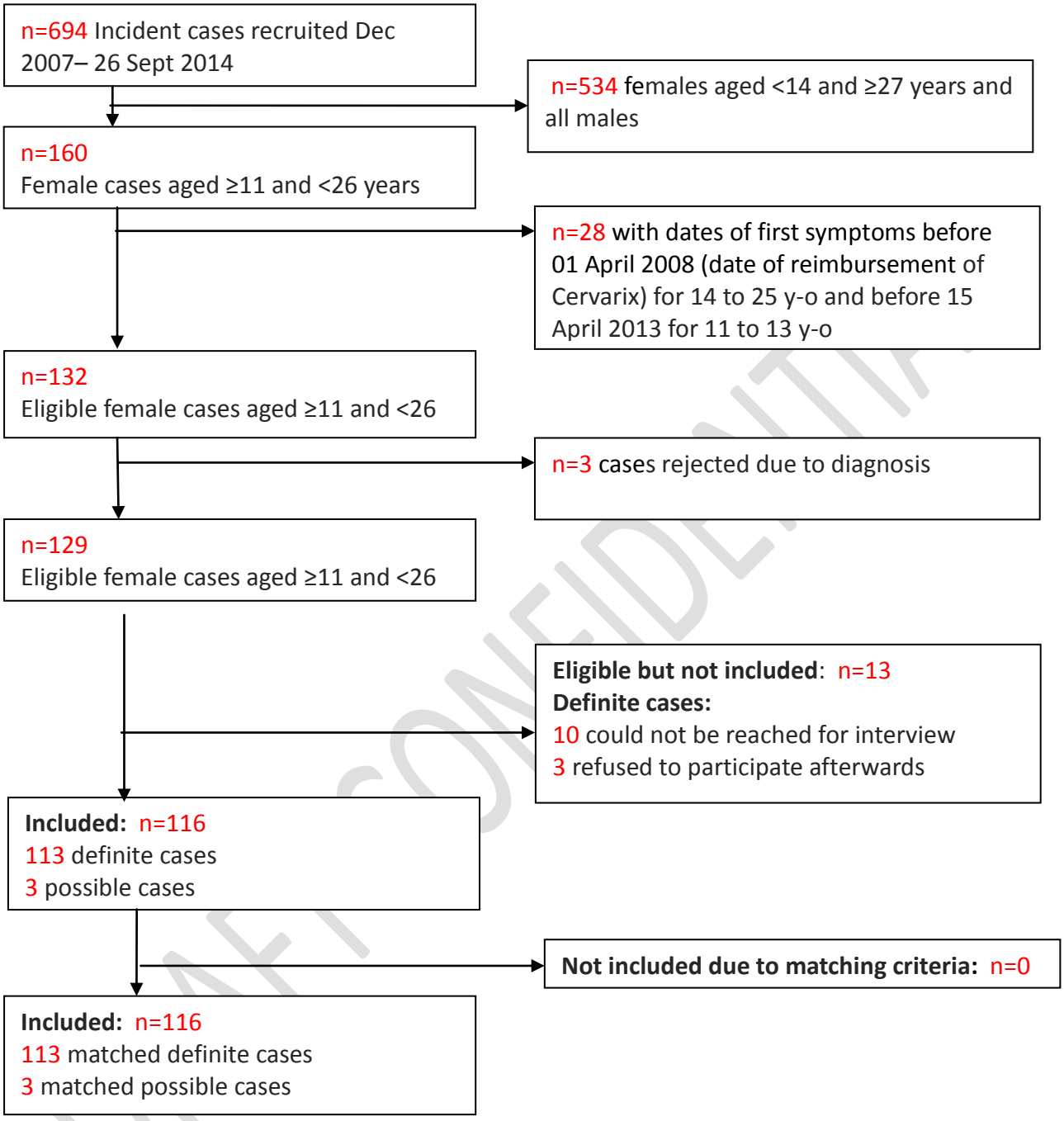


Figure 2. Flow chart showing identification of controls for central demyelination (CD/MS) cases within the pool of referents

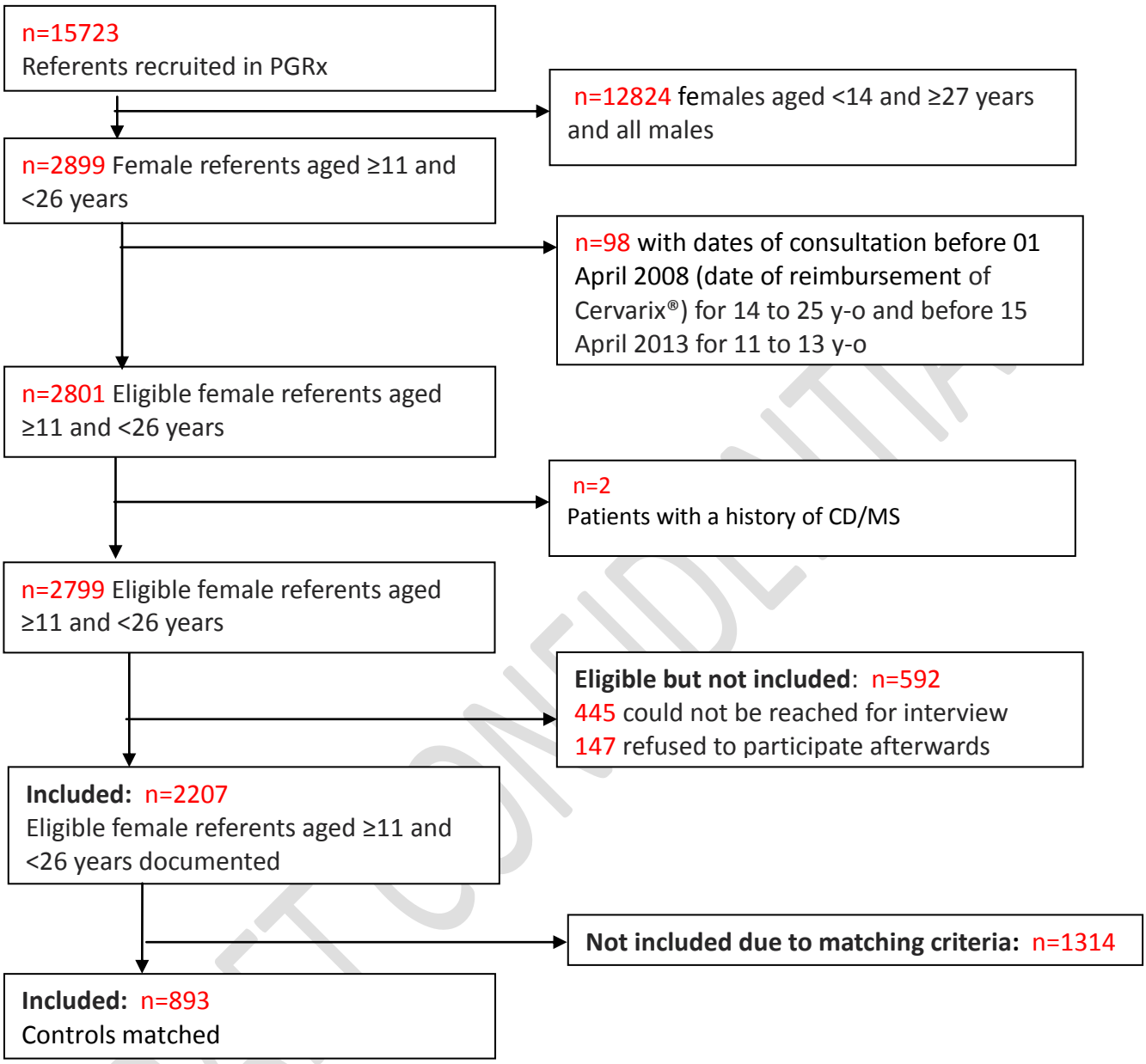
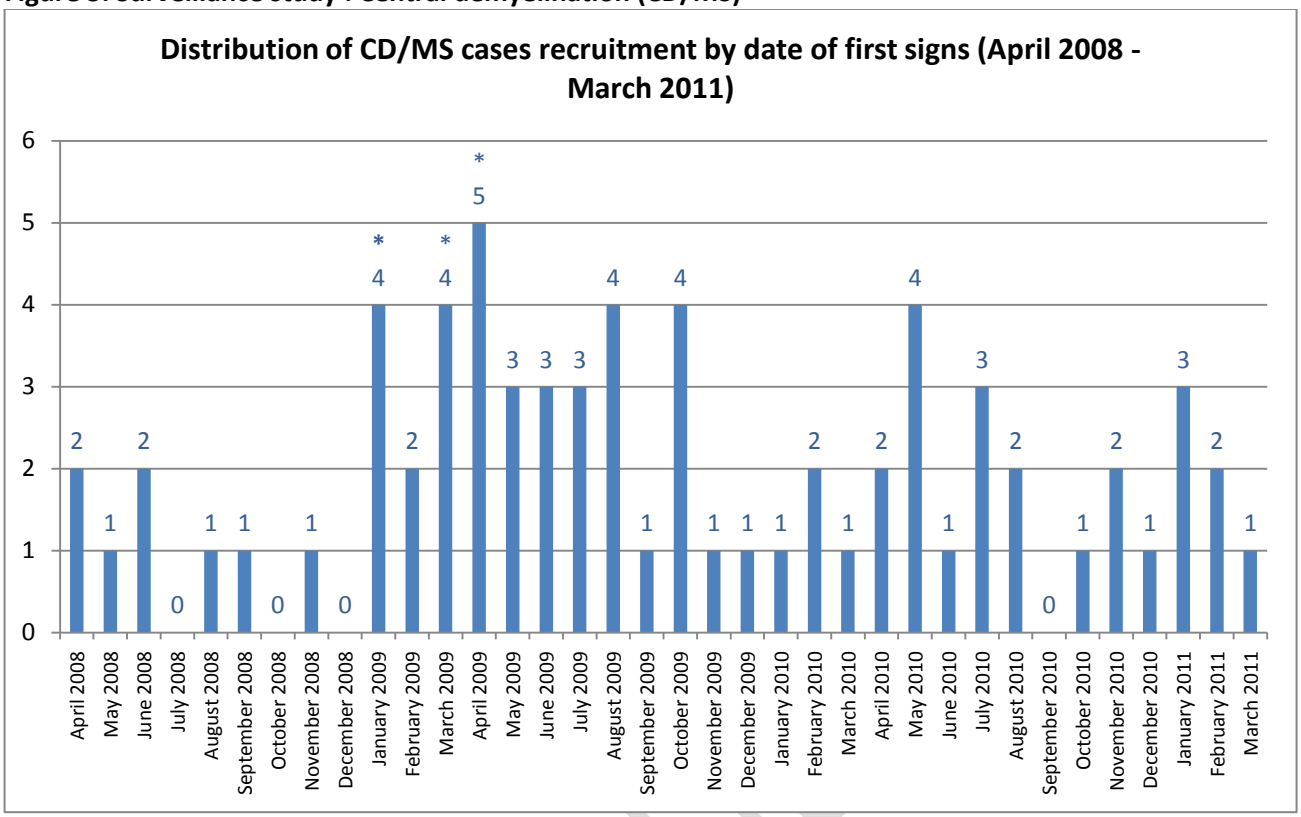
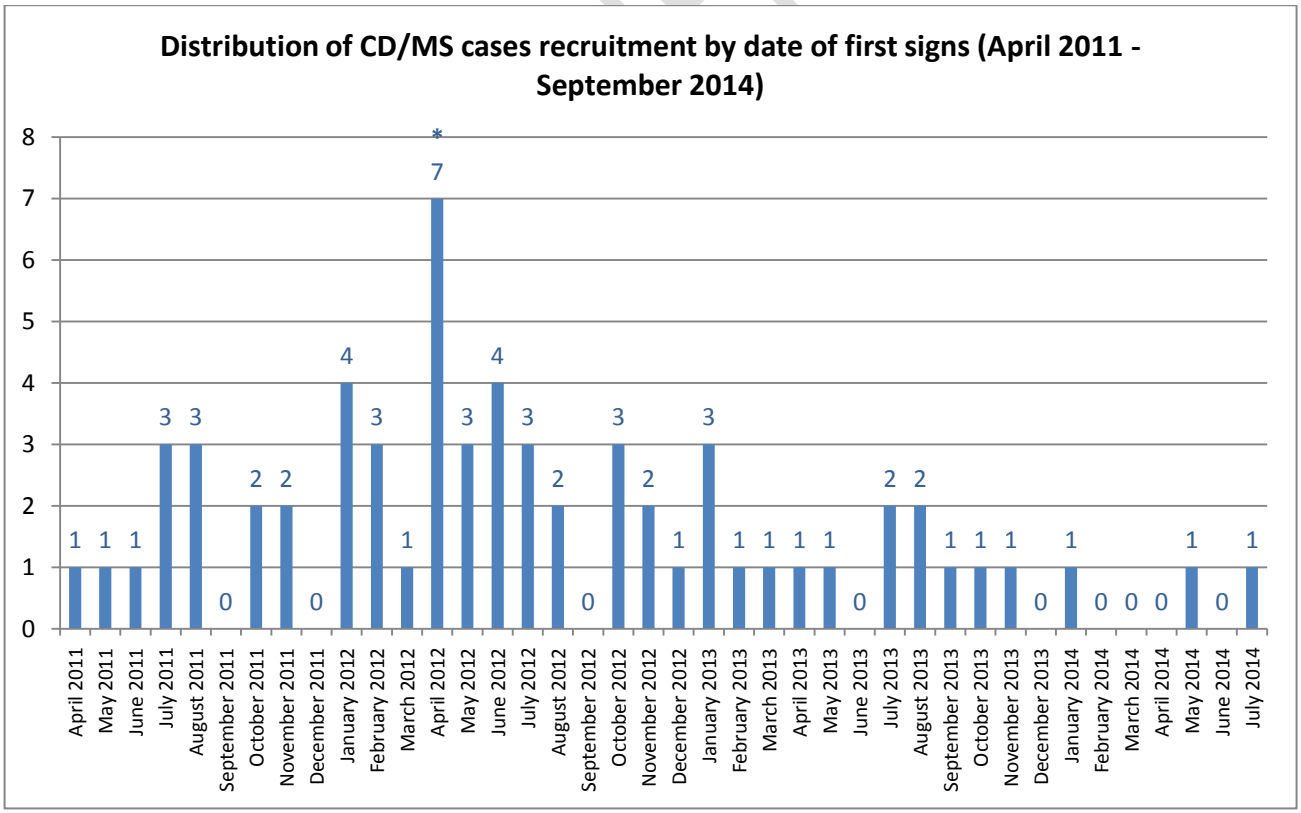


Figure 3. Surveillance study : Central demyelination (CD/MS)





Model 1: 1 peak (January 2009) were significantly different from the 3 preceding months.
No patient reported an HPV vaccination out of the 4 that occurred in January 2009.
Model 2 : 4 peaks (January, March and April 2009 and April 2012) were significantly different from the preceding months. In 2009, no patient reported a HPV vaccination out of the 4, 4 and 5 in January, March

and April respectively. In April 2012, one patient reported an HPV vaccination out of the 7 CD/MS cases that had occurred that month. None reported Cervarix® use.

Appendix – Connective Tissue Diseases (CTD) (Group 2)

Table 1. Case definition

Case definitions for the study of inflammatory arthritis

	Clinical presentation	Auto antibodies and imaging
Definite cases	At least 3 of the following criteria : ▪ Inflammatory pain chronology ▪ More than 2 joints with synovitis and/or pain ▪ Involvement of joints of the hand ▪ Bilateral disorders AND disorder present for at least 6 weeks	With or without anticitrullinated peptide antibodies With or without Rheumatoid factor With or without radiological articulation destruction
Possible cases	At least 2 of the 4 criteria above AND disorder present for at least 6 weeks	With or without anticitrullinated peptide antibodies With or without Rheumatoid factor With or without radiological articulation destruction

Case definitions for the study of lupus systemic disorder

	Number of clinical and-biological criteria from the ACR classification (except immunological disorders)	Lupus specific auto-antibodies: Anti-Sm, anti-DNA.
Definite cases	≥ 2 criteria	AND: - Anti-Sm, - OR anti-DNA - OR FAN
Possible cases	1 criterion	AND: - Anti-Sm, - OR anti-DNA - OR FAN

Case definitions for the study of cutaneous lupus

	Clinical picture	Lupus specific auto-antibodies (AAc)	Skin biopsy
Definite cases	Characteristic skin disorders: discoid lupus, lupus tumidus, annular lupus, Chilblain lupus, lupus profondus with or without systemic(s) disorder(s) evocative(s) of lupus	AND presence or absence of lupus specific AAc	AND biopsy performed with characteristic elements for lupus diagnosis OR biopsy not performed
Possible cases	Non characteristic skin disorder AND presence of systemic(s) disorder(s) evocative(s) of lupus	AND Absence of lupus specific AAc	AND biopsy performed but without characteristic elements for lupus diagnosis OR not performed

Rejected cases	Non characteristic skin disorder AND no systemic disorder evocative of lupus	AND Absence of lupus specific AAc	Not performed OR performed but without characteristic elements for lupus diagnosis

Case definitions for the study of incident myositis or dermatomyositis evocative disorders (Adapted from Hoogendijk, 2004)

	Arguments
Definite cases	Proximal Weakness OR Pain OR specific (eyelids heliotrope rash, hands Gottron papules) rash AND Raised CPK blood levels OR positive EMG AND Positive biopsy, inflammation OR necrosis Accepted Diseases - Polymyositis (pure) - Polymyositis associated with arthritis, interstitial lung disease, Raynaud phenomenon, lupus, or scleroderma anti-synthetase (OR anti-SRP OR anti-Mi2) antibodies - Dermatomyositis
Possible cases	Proximal Weakness OR Pain Biopsy with normal standard optic microscopy (AND Widespread muscle cells HLA-class I molecules hyperexpression OR C5b-9 muscle capillary deposits)
Rejected cases	Paraneoplastic myositis Cancer

Figure 1. Flow chart showing recruitment of connective tissue disease (CTD) cases used in the combined analysis

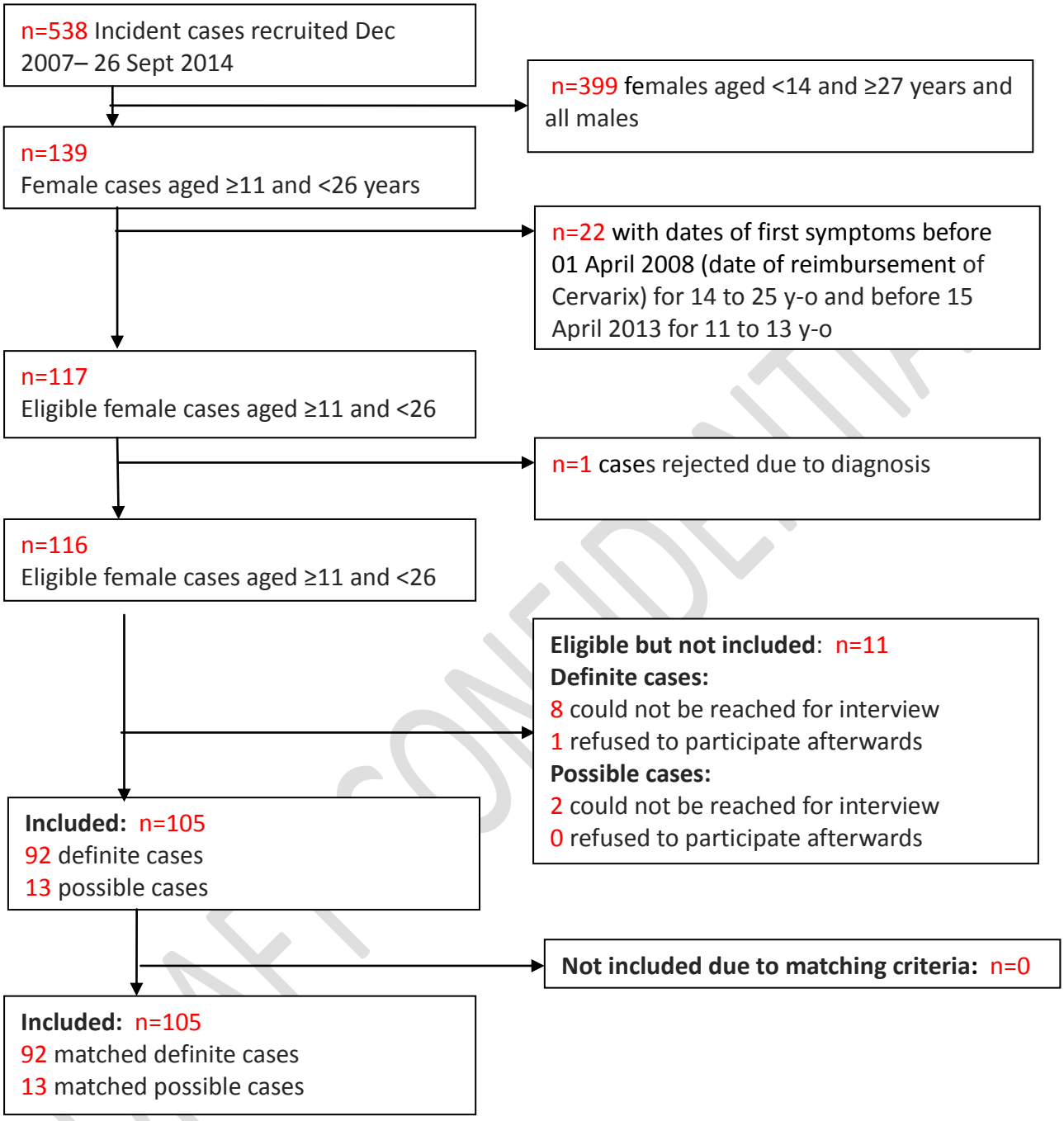


Figure 2. Flow chart showing identification of controls for connective tissue disease (CTD) cases within the pool of referents

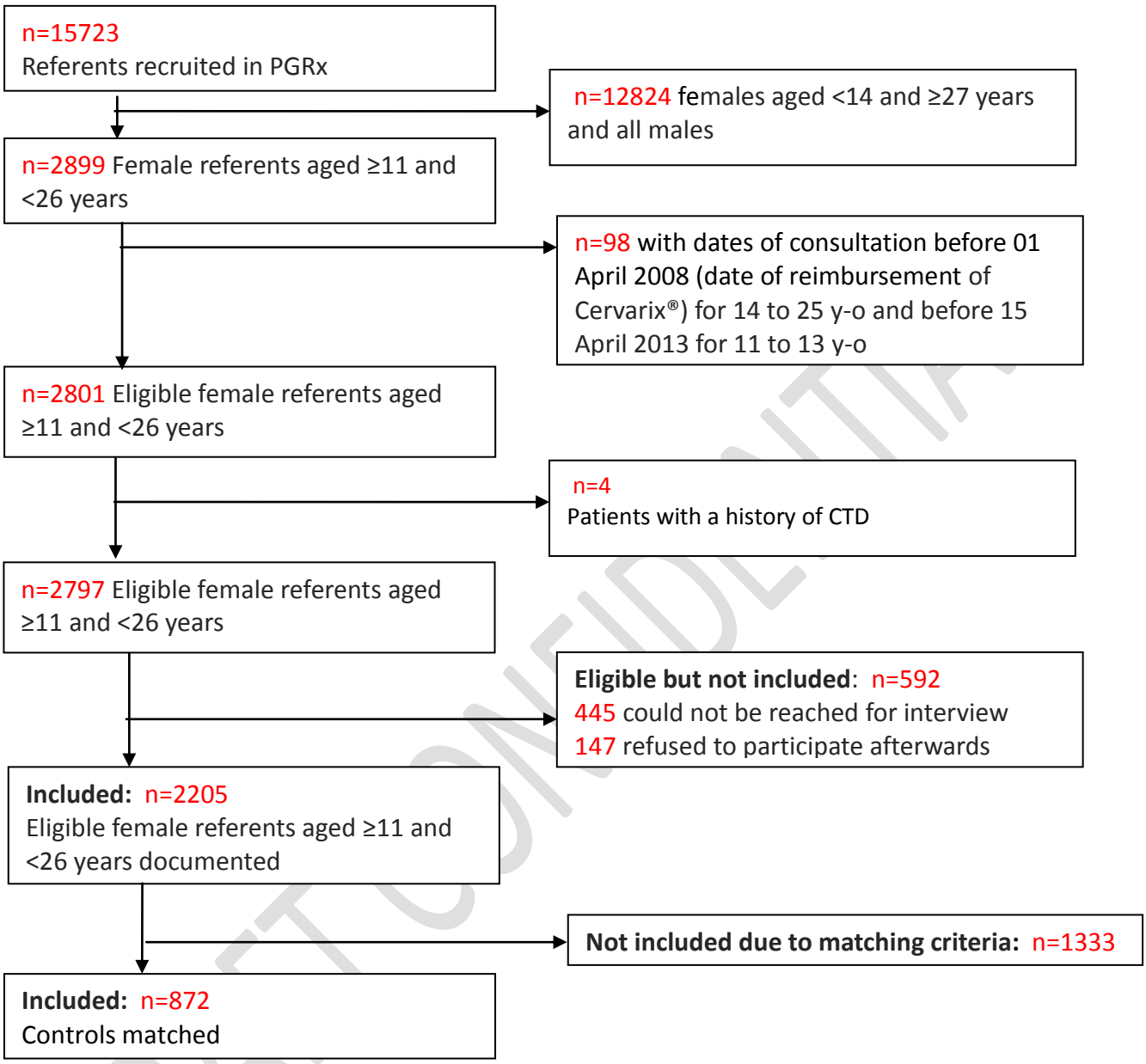
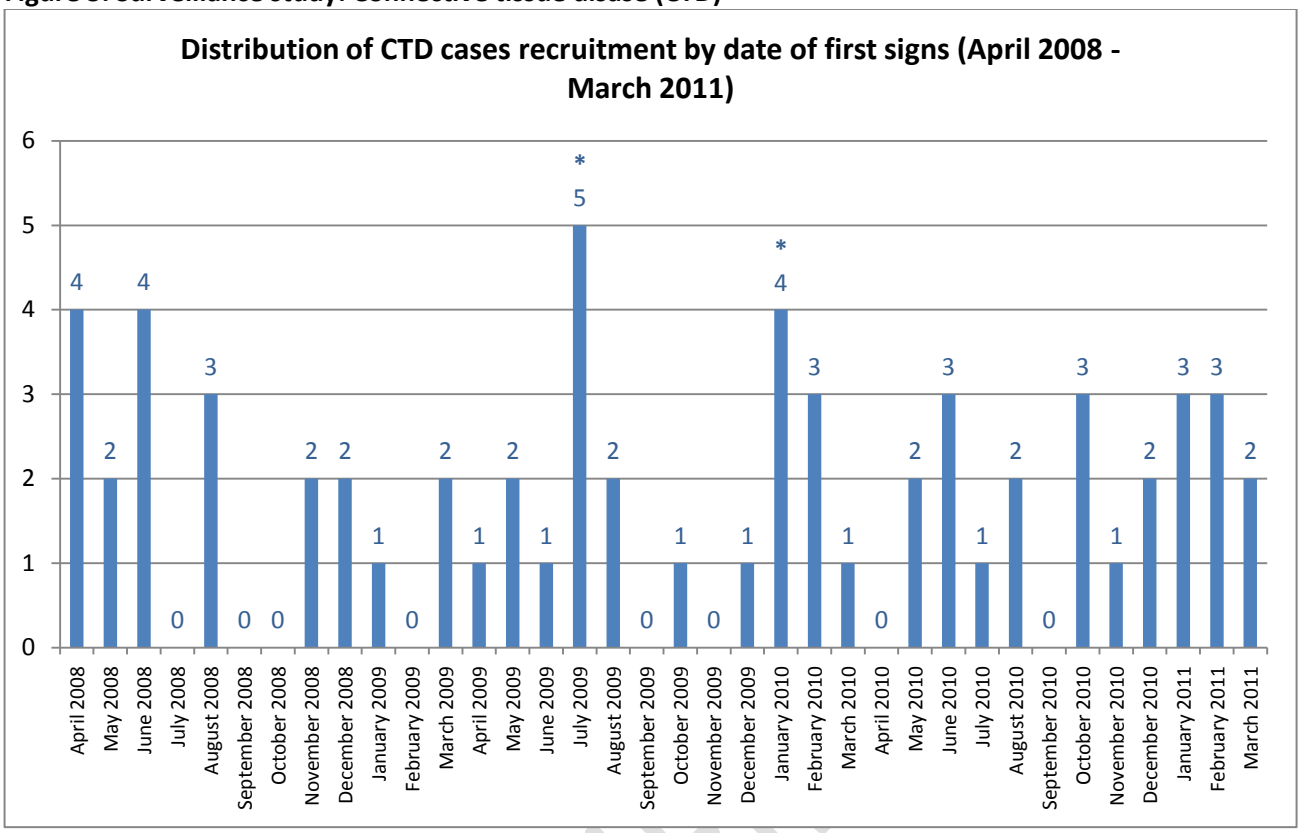
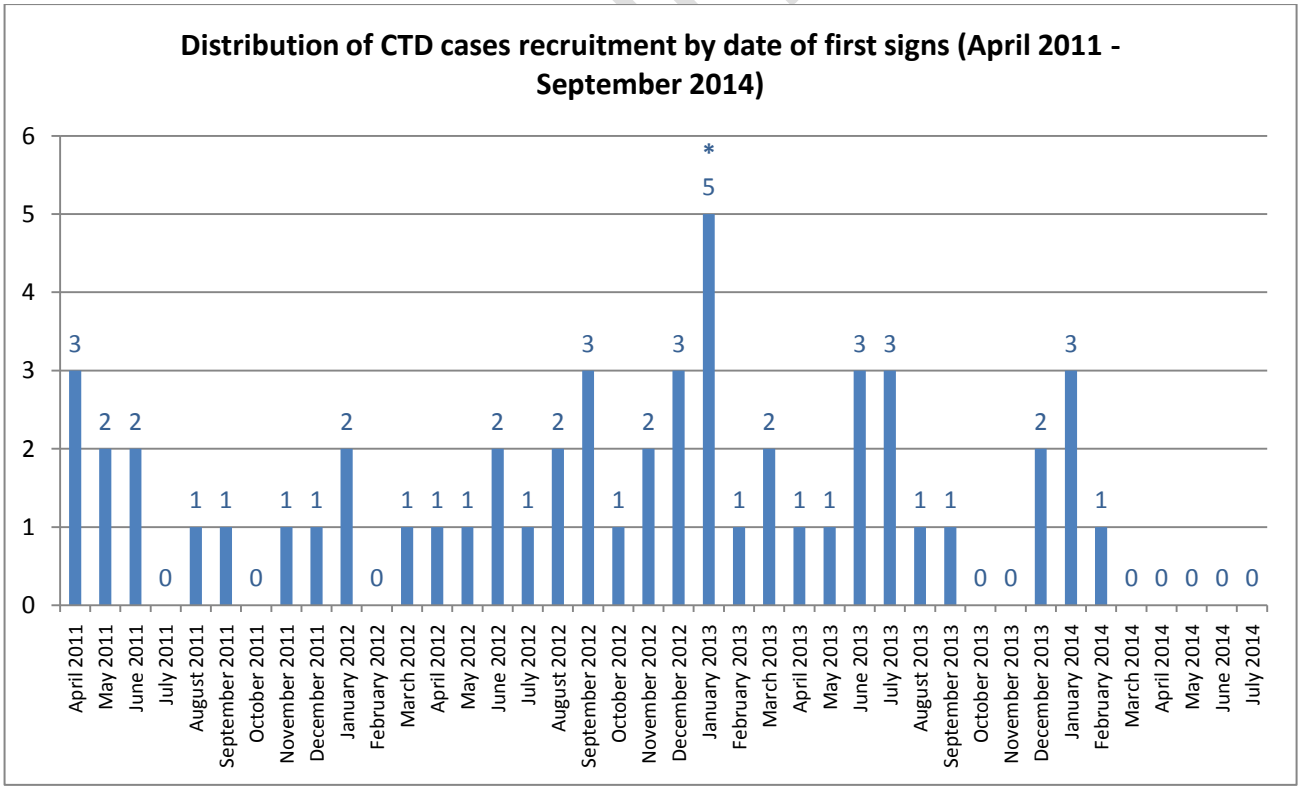


Figure 3. Surveillance study: Connective tissue disase (CTD)





Model 1: 2 peaks (July 2009 and January 2010) were significantly different from the 3 preceding months. In July 2009, no patient reported an HPV use. In January 2010 one patient reported an HPV vaccination out of the 4 CTD cases that had occurred that month. None reported Cervarix® use.
Model 2: 2 peaks (July 2009 and January 2013) were significantly different from the preceding months.

In July 2009, no patient reported a HPV use. In January 2013 three patients reported an HPV vaccination out of the 5 CTD cases that had occurred that month. None reported Cervarix® use.

Appendix – Guillain-Barré Syndrome (Group 3)

Table 1. Case definition

Case definition for the study according to the Brighton collaboration case definition

	Clinical presentation
Definite cases (Level 1)	Requires clinical, electrophysiologic, and CSF data consistent with the onset of GBS
Probable cases (Level 2)	Requires clinical data and either electrophysiologic, OR CSF data consistent with the onset of GBS
Possible cases (Level 3)	Requires clinical data consistent with the onset of GBS

Figure 1. Flow chart showing recruitment of Guillain-Barre Syndrom (GBS) cases used in the combined analysis

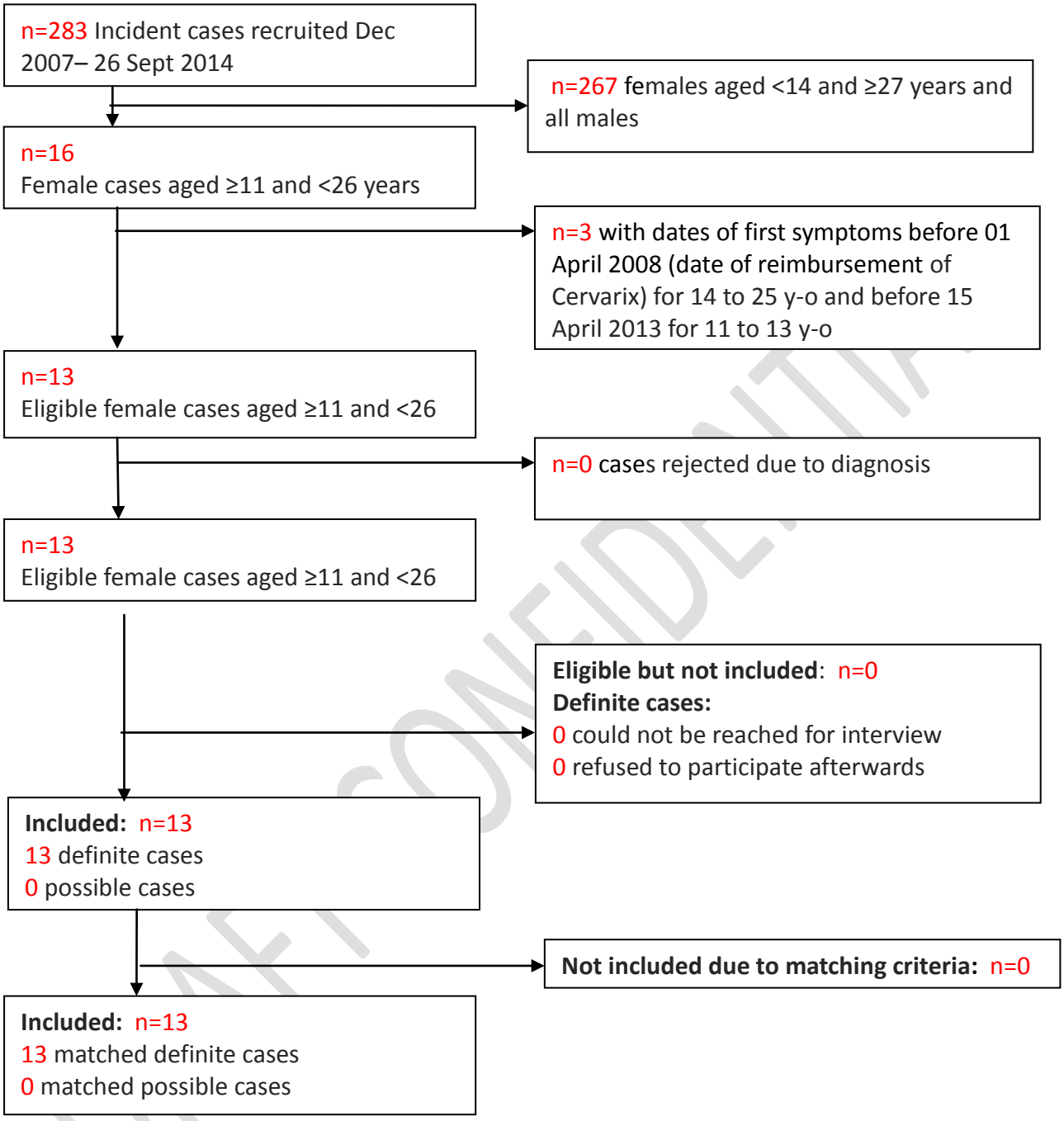


Figure 2. Flow chart showing identification of controls for Guillain-Barre Syndrom (GBS) cases within the pool of referents

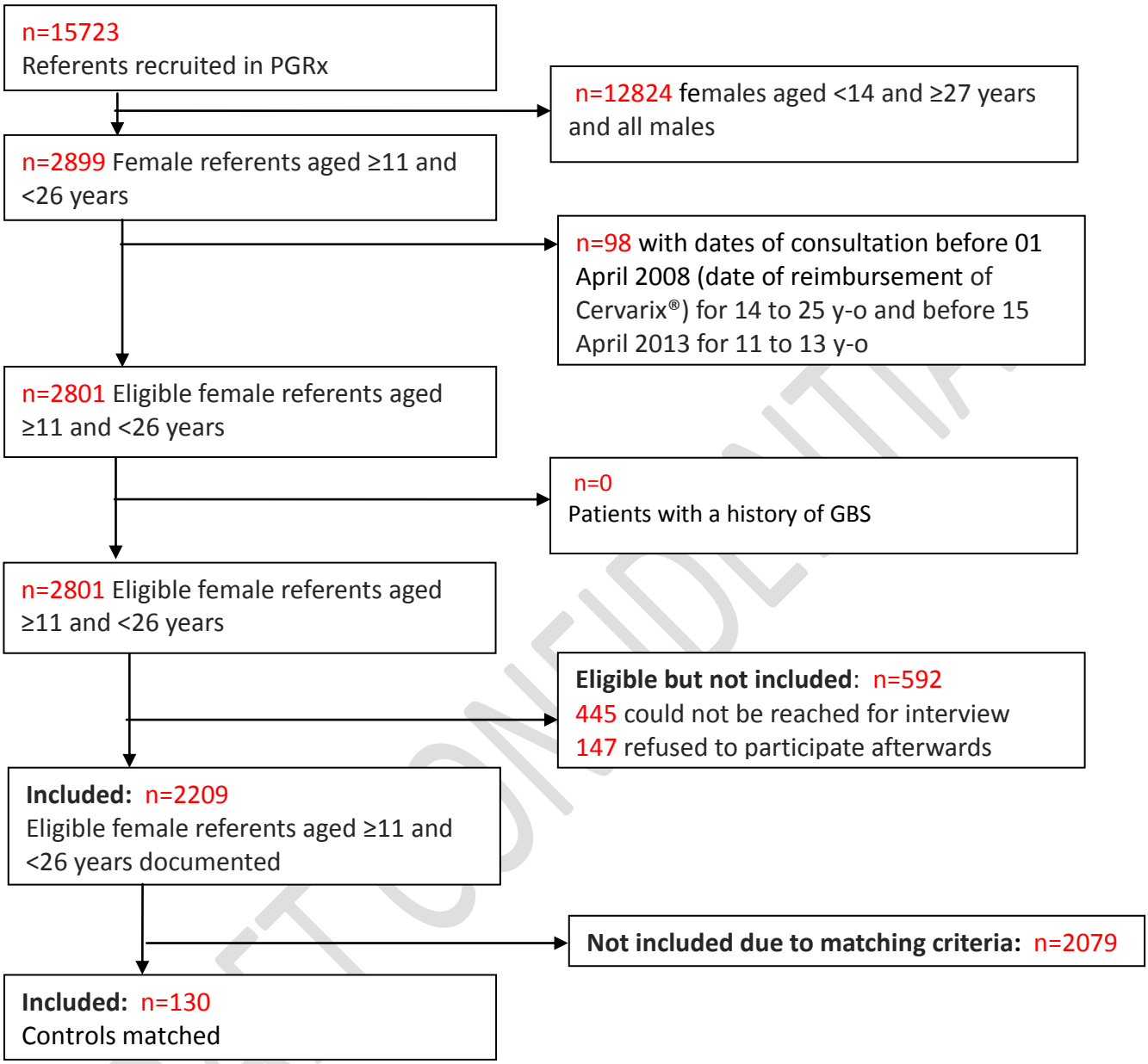
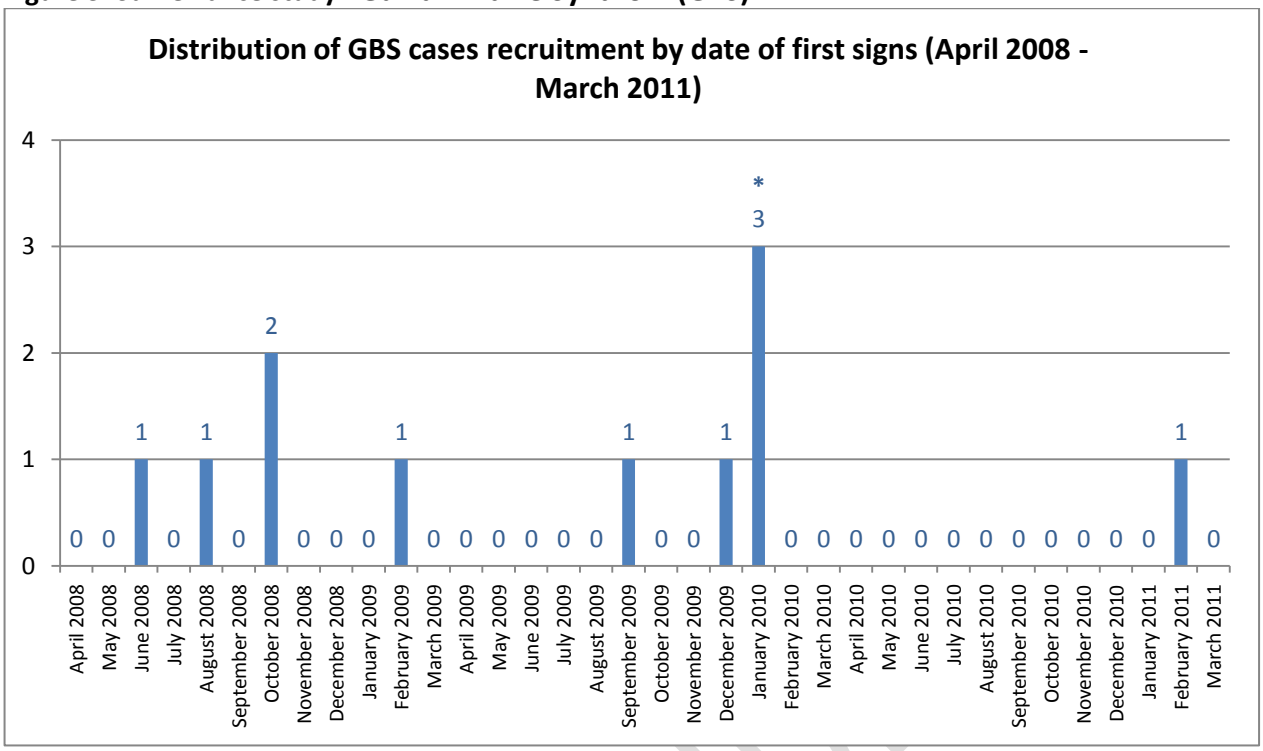
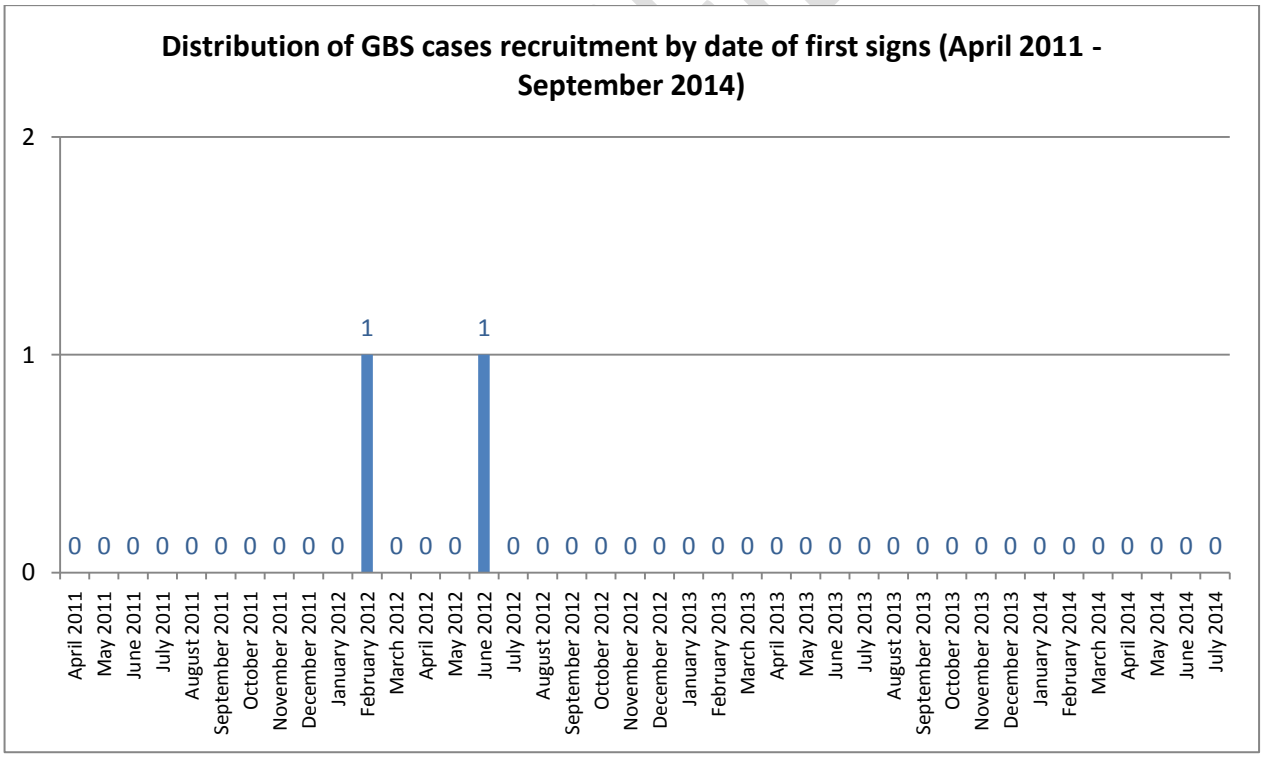


Figure 3. Surveillance study : Guillain-Barre Syndrom (GBS)





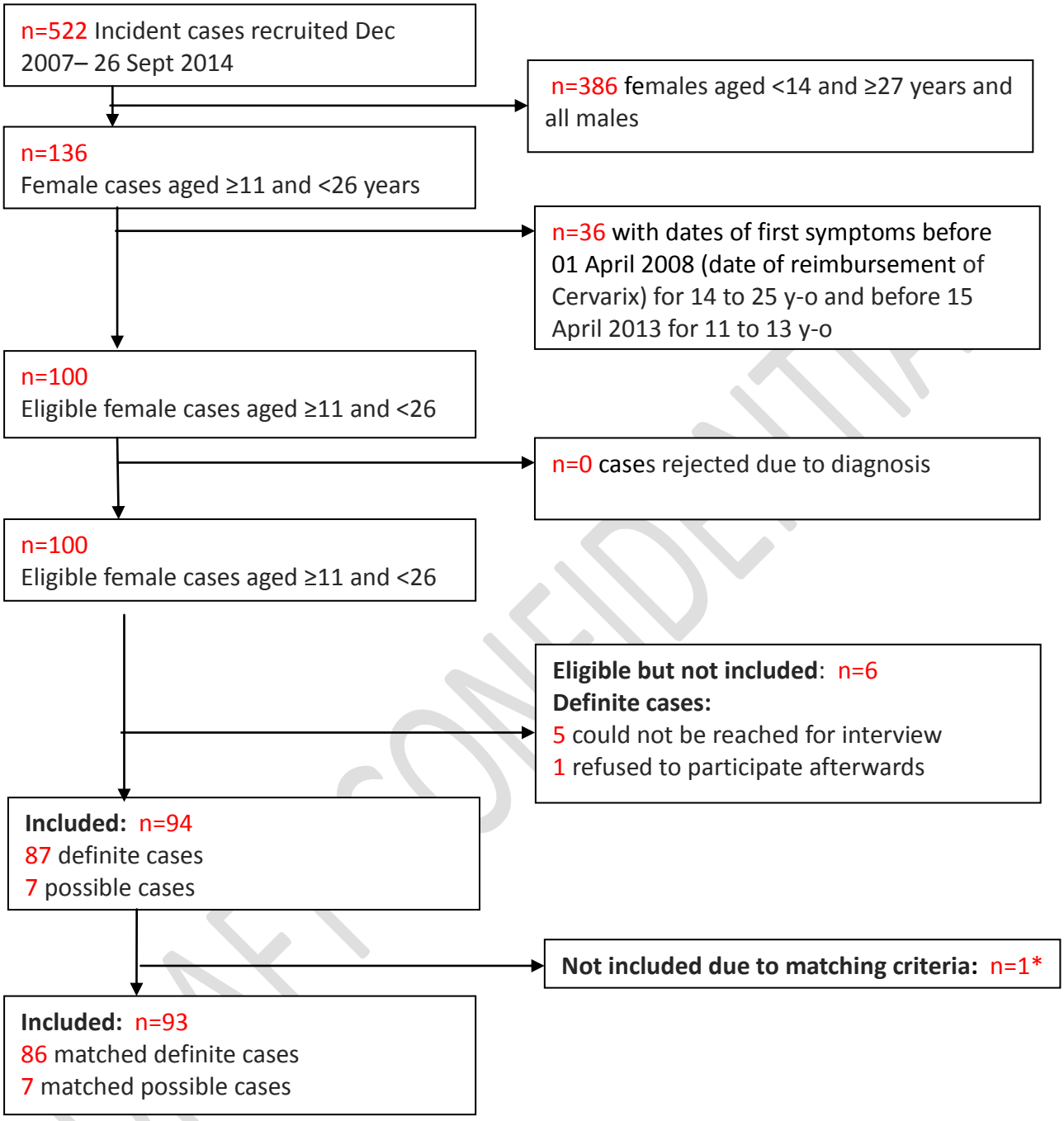
Model 1: No peak
Model 2 : 1 peak (January 2010) was significantly different from the preceding months.
In January 2010, no patient reported an HPV vaccine use.

Appendix – Type I diabetes (Group 4)

Table 1. Case definition
Case definitions for the study of type 1 diabetes

	Clinical presentation	Biological tests	Auto-antibodies (AAb)
Definite cases	Abrupt onset with polyuro-polydipsia AND/OR weight loss AND/OR asthenia AND/OR acido-cetosis	AND Hyperglycemia > 2g/l Glycosuria	AND Presence of anti-insuline, or anti-GAD, or anti-IA2
Possible cases		Hyperglycemia > 2g/l Glycosuria	No AAb

Figure 1. Flow chart showing recruitment of Type I diabetes (TID) cases used in the combined analysis



*This case could also not be matched for the study all combined AID, female T1D case aged 11.2 year-old categorised as definite case.

Figure 2. Flow chart showing identification of controls for Type I diabetes (TID) cases within the pool of referents

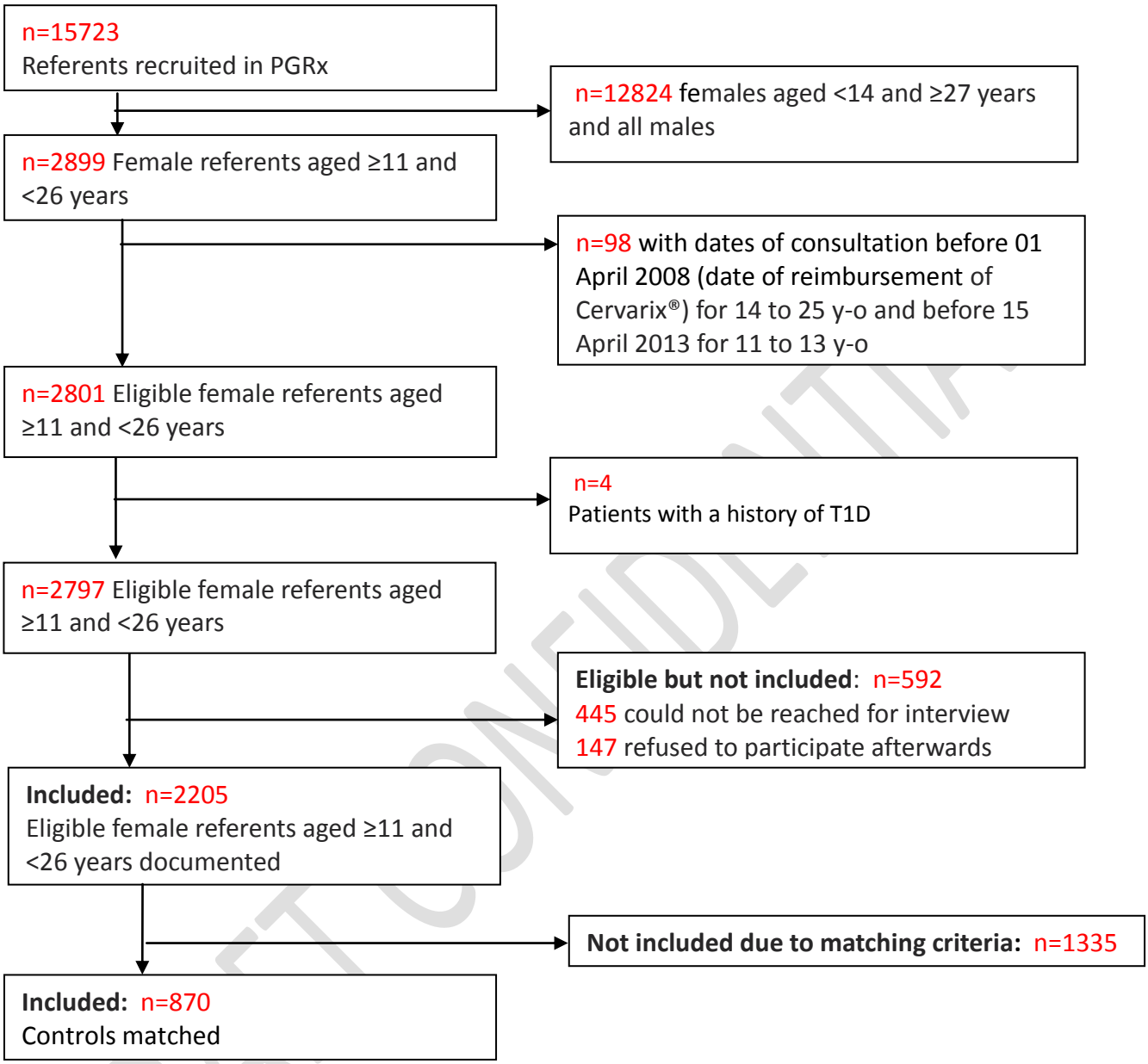
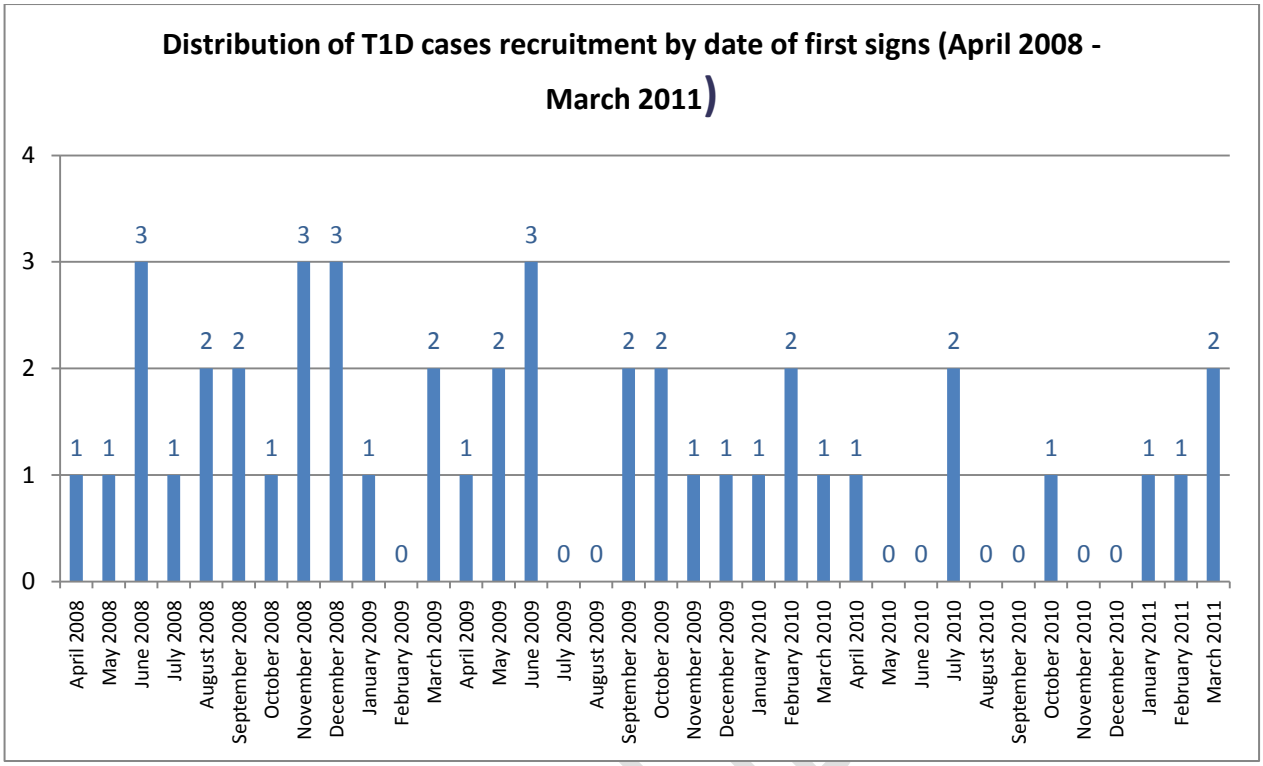
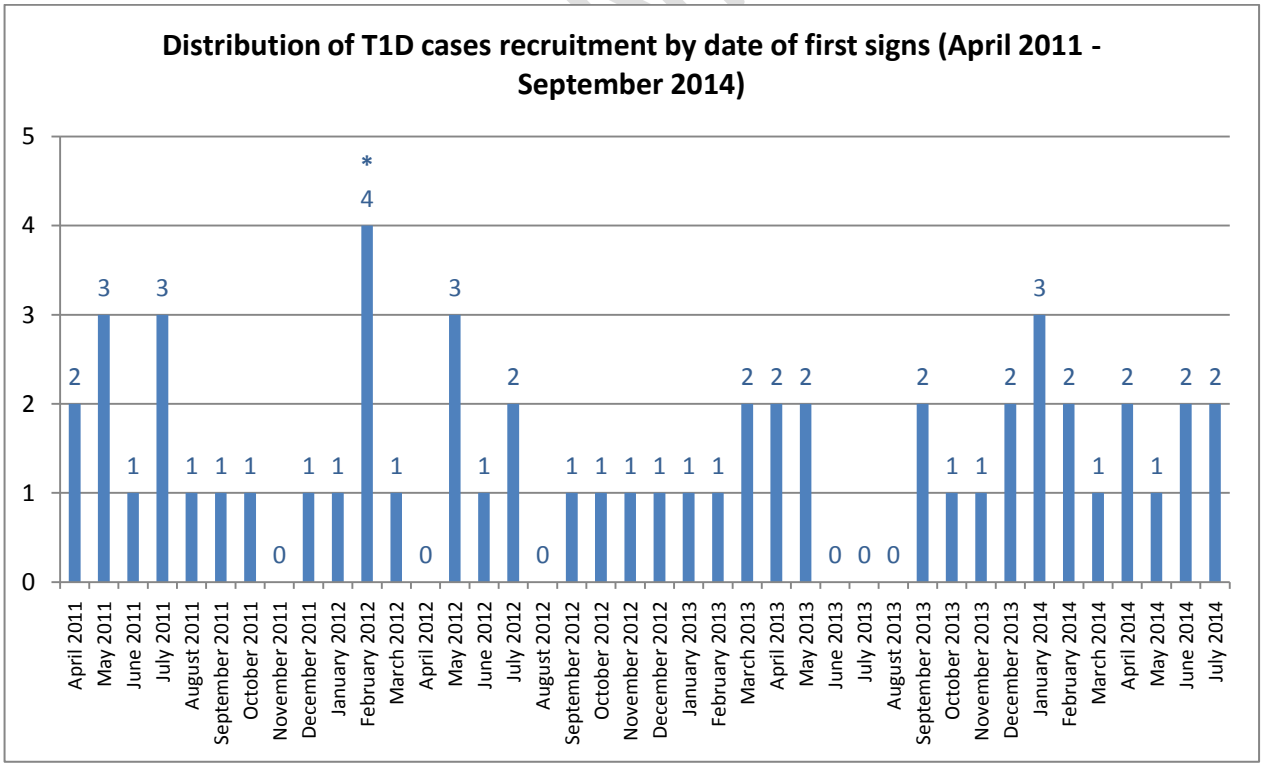


Figure 3. Surveillance study : Type I diabetes (TID)





Model 1 & Model 2 : 1 peak (February 2012) was significantly different from the comparator in both models.
In February 2012, no patient reported an HPV use.

Appendix – Autoimmune thyroiditis (Group 4)

Table 1. Case definition

Case definitions for the study of incident auto-immune thyroiditis evocative disorders

	Clinical presentation	Biologic examinations
Definite cases	Hypothyroidism consistent with incident auto-immune thyroiditis	AND anti-peroxydase (anti-TPO) AND increased TSH > 7 mU/L
Possible cases		AND anti-thyroglobuline (anti-TG) AND 4 mU/L< TSH < 7 mU/L
OR *Subclinical thyroiditis*	Discrete symptoms or absence of symptoms AND thyroid gland with normal or borderline size	AND decreased TSH AND anti-peroxydase (anti-TPO) AND/OR Anti-thyroglobulin (anti-TG)

Case definitions for the study of incident Graves's disease evocative disorders

	Thyrotoxicosis	Thyroid gland	Auto-antibodies	TSH
Definite cases	Presence of exophthalmia or palsy or tachycardia or weight loss or weight gain	-	AND anti-TSH-receptor	AND decreased TSH

Figure 1. Flow chart showing recruitment of Autoimmune thyroiditis (AIT) cases used in the combined analysis

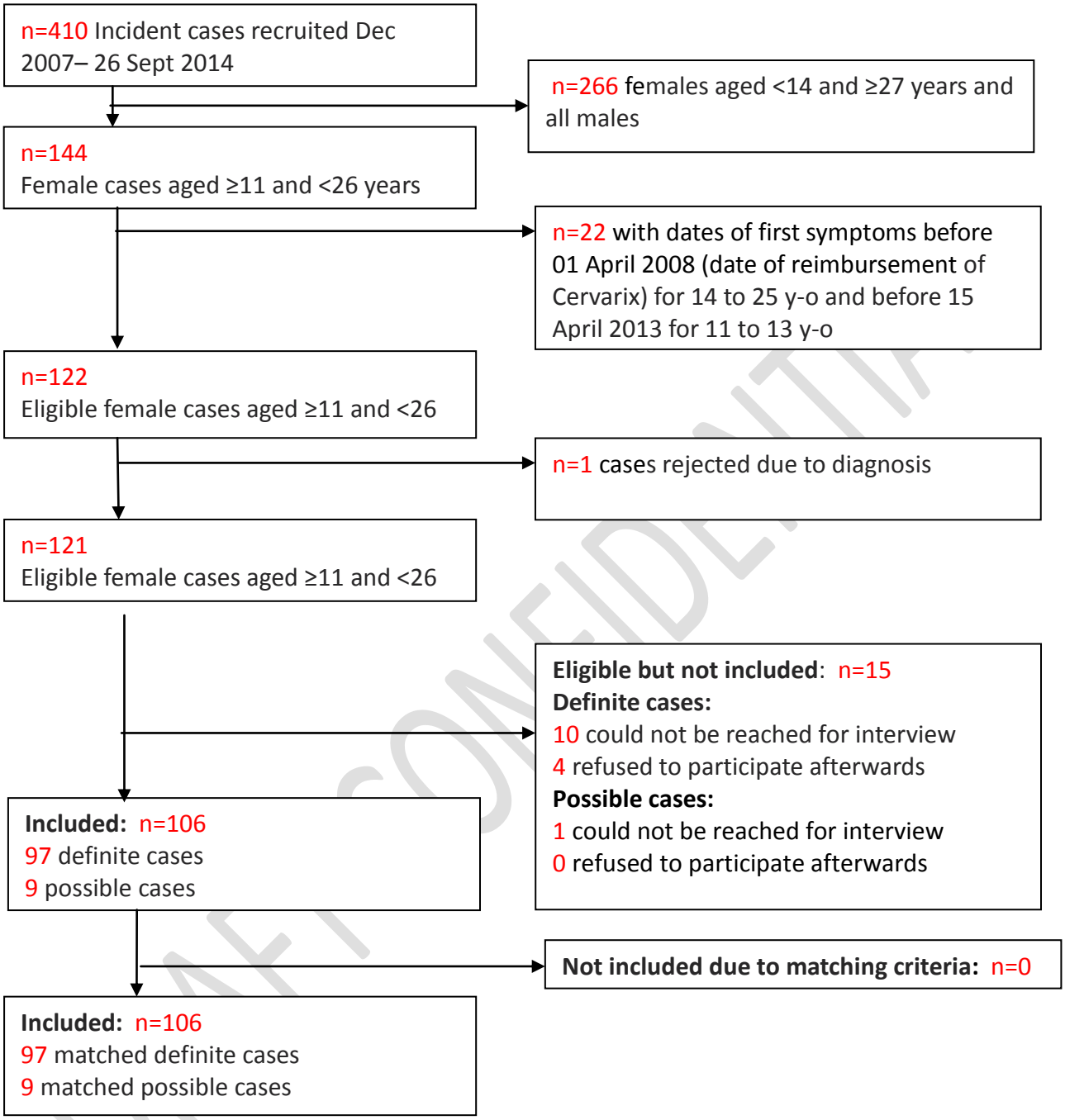


Figure 2. Flow chart showing identification of controls for Autoimmune thyroiditis (AIT) cases within the pool of referents

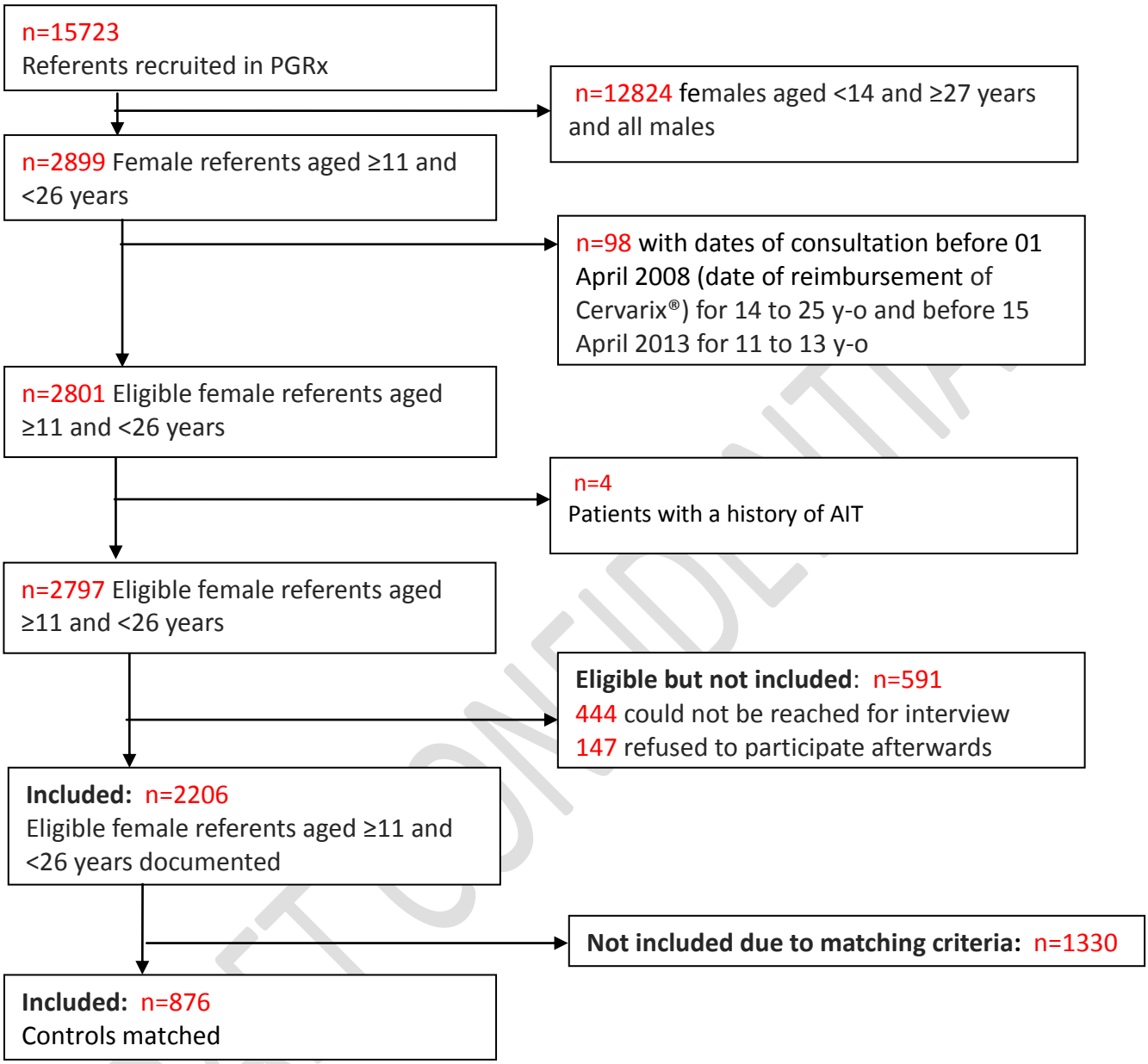
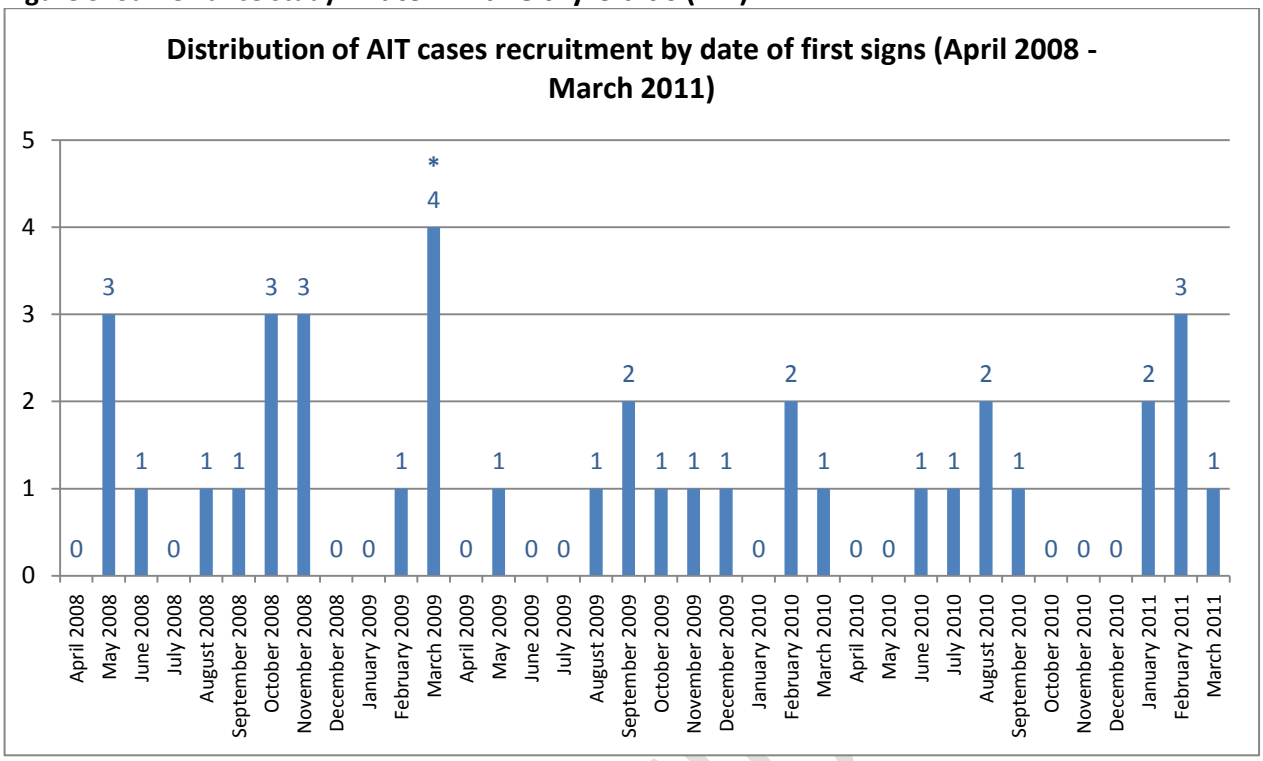
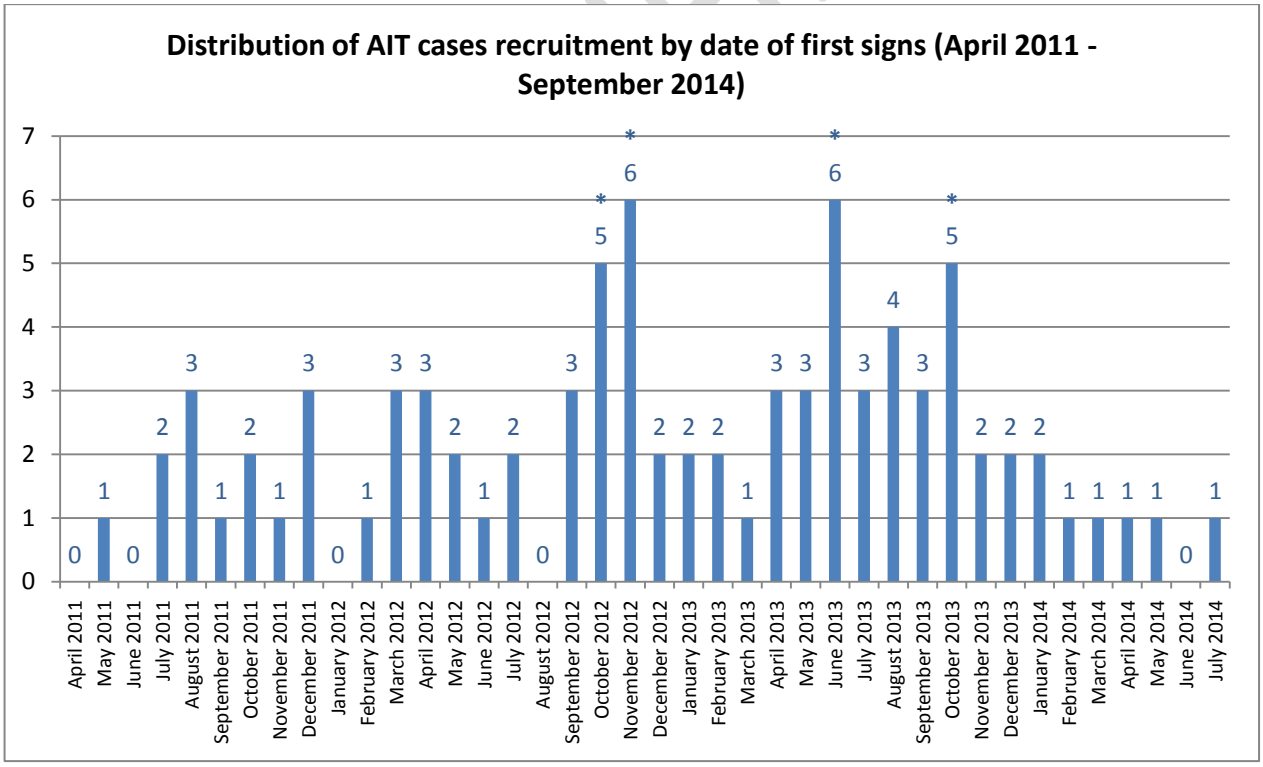


Figure 3. Surveillance study : Autoimmune thyroiditis (AIT)





Model 1: 1 peak (March 2009) were significantly different from the 3 preceding months.
In March 2009, no patient reported an HPV use.
Model 2 : 5 peaks (March 2009, October 2012, November 2012, June 2013 and October 2013) were significantly different from the preceding months.
In March 2009, no patient reported an HPV use. In October 2012, November 2012, and June 2013 two patients reported an HPV vaccination out of the 5, 6 and 6 AIT cases that had occurred in the respective

months. In October 2013, three patients reported an HPV vaccination out of the 5 AIT cases that had occurred that month. None reported Cervarix® use.

Appendix – Immune Thrombocytopenic Purpura (Group 5)

Table 1. Case definition
Case definitions for the study of immune thrombocytopenic purpura

	Elements for definition		
	Normal platelet count in the previous 12 months	Clinical signs	Platelet count at inclusion
Definite cases	Documented	Acute hemorrhage syndrome	≤ 50.10^9/L
	Documented	Fortuitous discover of thrombocytopenia	≤ $50. 10^9$/L
Possible cases	Not documented	Acute hemorrhage syndrome	≤ $50. 10^9$/L
	Not documented	Fortuitous discover of thrombocytopenia	whatever the platelet count

Figure 1. Flow chart showing recruitment of Immune Thrombocytopenic Purpura (ITP) cases used in the combined analysis

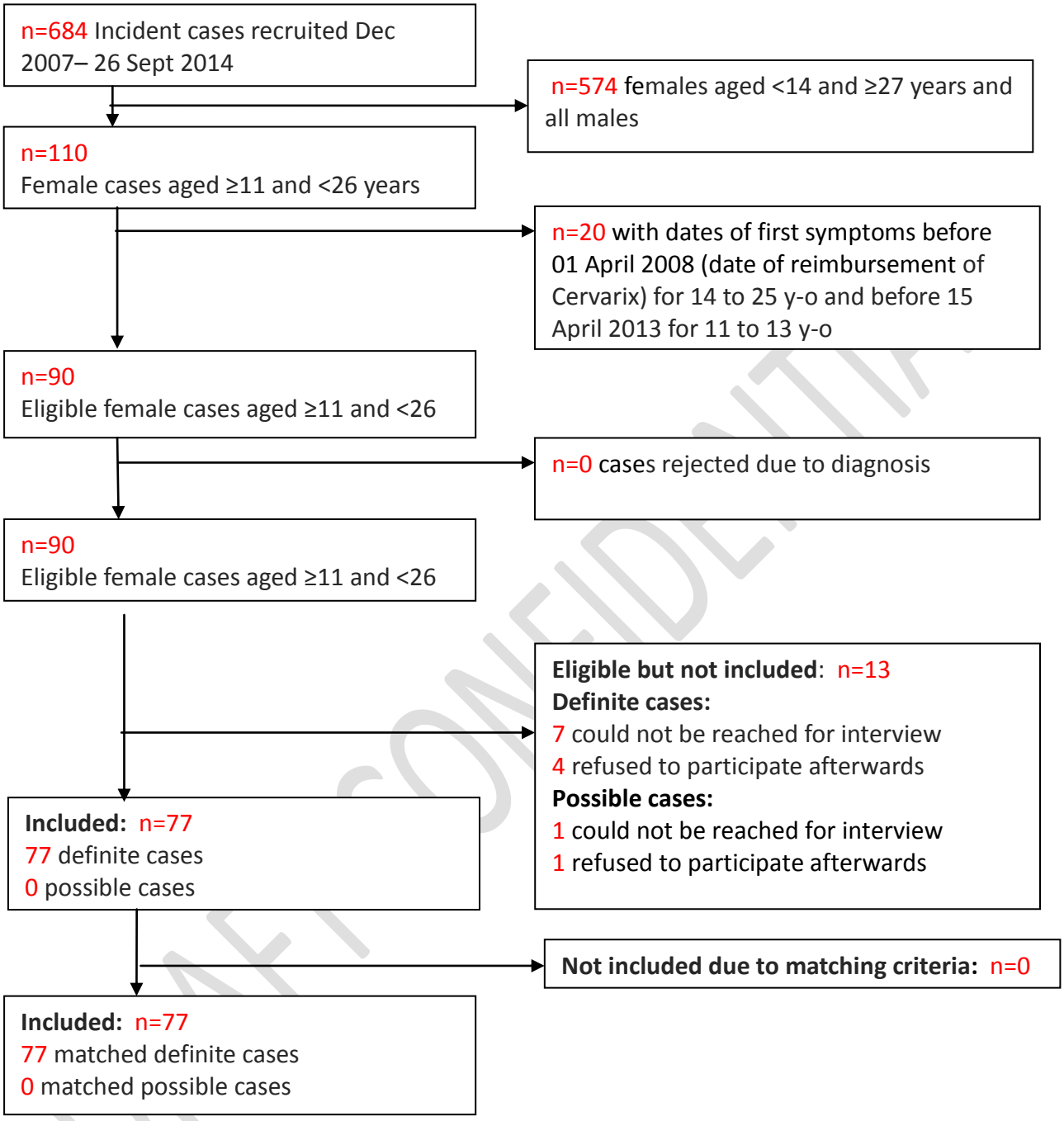


Figure 2. Flow chart showing identification of controls for Immune Thrombocytopenic Purpura (ITP) cases within the pool of referents

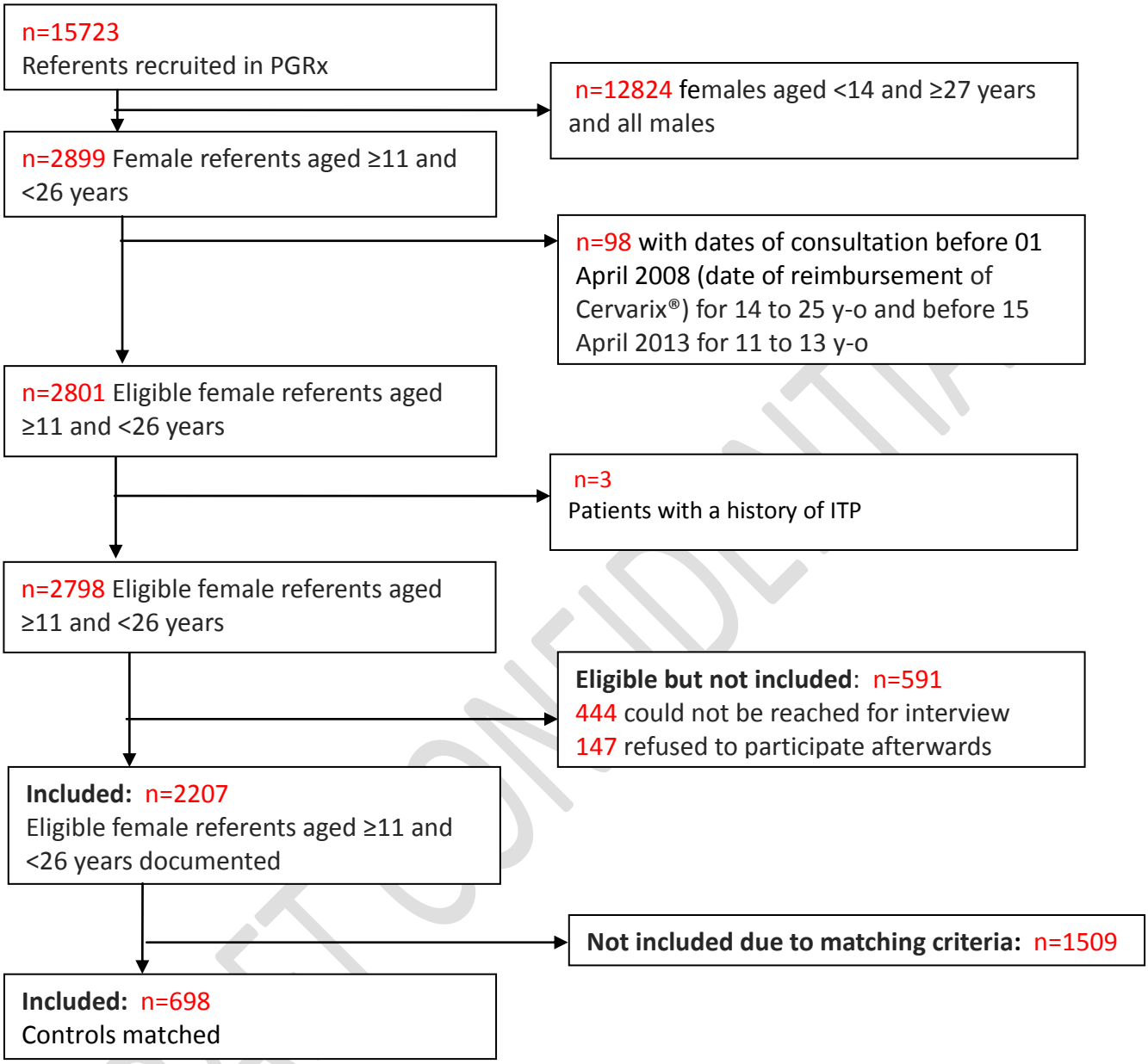
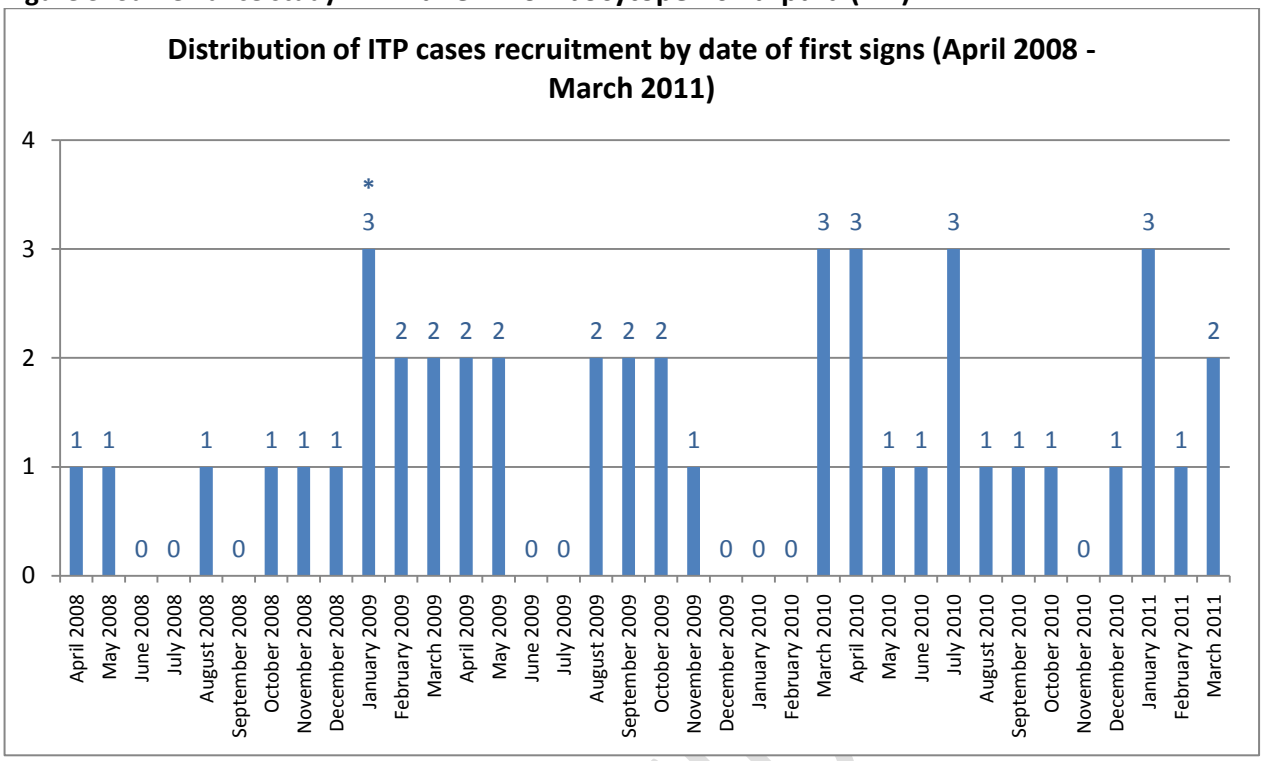
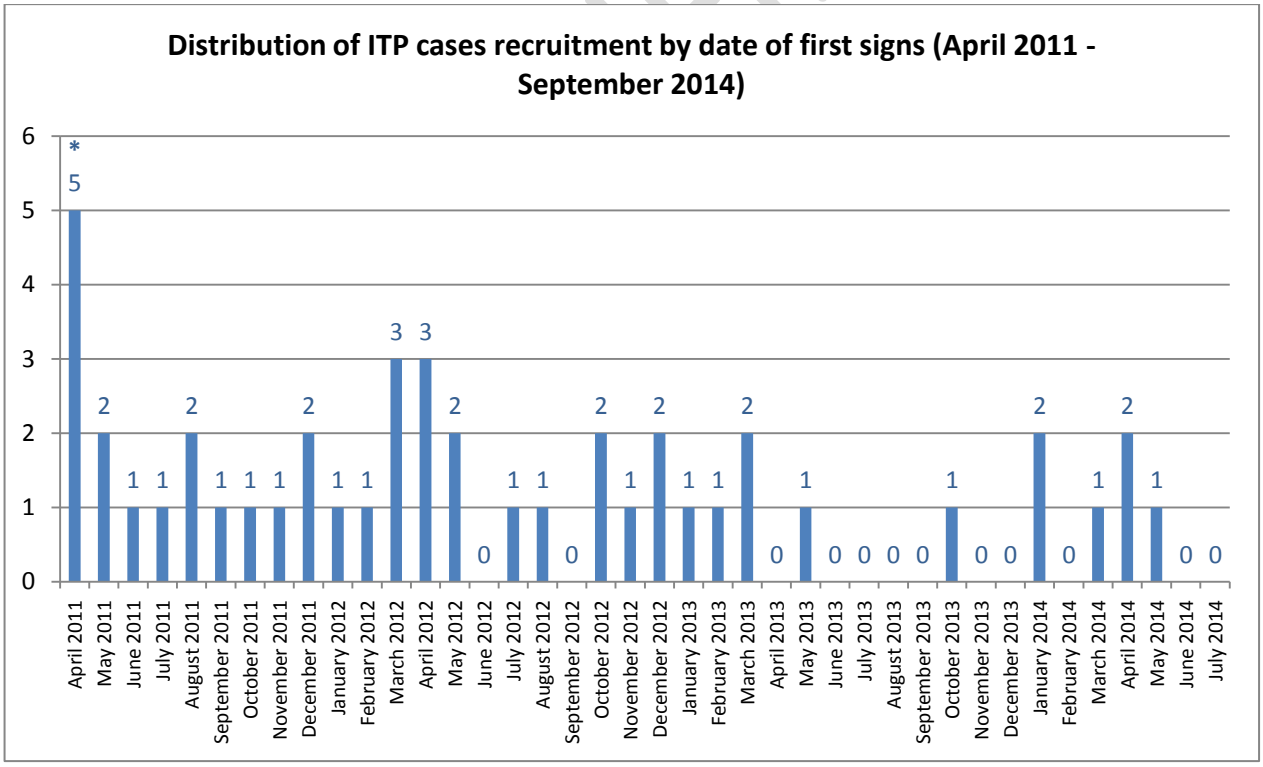


Figure 3. Surveillance study : Immune Thrombocytopenic Purpura (ITP)





Model 1: No peak

Model 2 : 2 peaks (January 2009 and April 2011) were significantly different from the preceding months. In January 2009, one patient reported an HPV vaccination out of the 3 ITP cases that had occurred that month. In April 2011, two patients reported an HPV vaccination out of the 5 ITP cases that had occurred that month. None reported Cervarix® use.

References

Miettinen OS, Caro JJ. Principles of nonexperimental assessment of excess risk, with special reference to adverse drug reactions.J Clin Epidemiol. 1989;42(4):325-31.

Schlesselman JJ. Sample size requirements in cohort and case-control studies of disease. Am J Epidemiol. 1974 Jun;99(6):381-4. Review.

Wacholder S, McLaughlin JK, Silverman DT, Mandel JS. Selection of controls in case-control studies. I. Principles. Am J Epidemiol. 1992 May 1;135(9):1019-28.

Walker AM, Lanes SF. Misclassification of covariates. Stat Med. 1991 Aug;10(8):1181-96.

Grimaldi-Bensouda L, Le Guern V, Kone-Paut I, Aubrun E, Fain O, Ruel M, Machet L, Viallard JF, Magy-Bertrand N, Daugas E, Rossignol M, Abenhaim L, Costedoat-Chalumeau N; PGRx Lupus Study Group. The risk of systemic lupus erythematosus associated with vaccines: an international case-control study. Arthritis Rheumatol. 2014 Jun;66(6):1559-67.

Grimaldi-Bensouda L, Alpérovitch A, Aubrun E, Danchin N, Rossignol M, Abenhaim L, Richette P; the PGRx MI Group. Impact of allopurinol on risk of myocardial infarction. Ann Rheum Dis. 2014 Jan 6.

Grimaldi-Bensouda L, Guillemot D, Godeau B, Bénichou J, Lebrun-Frenay C, Papeix C, Labauge P, Berquin P, Penfornis A, Benhamou PY, Nicolino M, Simon A, Viallard JF, Costedoat-Chalumeau N, Courcoux MF, Pondarré C, Hilliquin P, Chatelus E, Foltz V, Guillaume S, Rossignol M, Abenhaim L; PGRx-AID Study Group. Autoimmune disorders and quadrivalent human papillomavirus vaccination of young female subjects. J Intern Med. 2014 Apr;275(4):398-408.

Grimaldi-Bensouda L, Rossignol M, Danchin N, Dallongeville J, Bruckert E, Banayan J, Cottin Y, Aubrun E, Khachatryan A, Bénichou J, Abenhaim L; PGRx-MI study group. Real-life effectiveness of statins in the prevention of first acute coronary syndrome in France: a prospective observational study. Int J Cardiol. 2013 Nov 15;169(4):271-5.

Grimaldi-Bensouda L, Michel M, Aubrun E, Leighton P, Viallard JF, Adoue D, Magy-Bertrand N, Tisserand G, Khellaf M, Durand JM, Quittet P, Fain O, Bonnotte B, Morin AS, Limal N, Costedoat-Chalumeau N, Morel N, Pan-Petesch B, Decaux O, Mahevas M, Ruel M, Sacre K, Lefrere F, Abenhaim L, Godeau B; PGRx Immune Thrombocytopenia Study Group. A case-control study to assess the risk of immune thrombocytopenia associated with vaccines. Blood. 2012 Dec 13;120(25):4938-44.

Grimaldi-Bensouda L, Alpérovitch A, Besson G, Vial C, Cuisset JM, Papeix C, Lyon-Caen O, Benichou J, Rossignol M; Lucien Abenhaim for the GBS-PGRx Study Group. Guillain-Barre syndrome, influenzalike illnesses, and influenza vaccination during seasons with and without circulating A/H1N1 viruses. Am J Epidemiol. 2011 Aug 1;174(3):326-35.

Grimaldi-Bensouda L, Aubrun E, Leighton P, Benichou J, Rossignol M, Abenhaim L; PGRx Study Group. Agreement between patients' self-report and medical records for vaccination: the PGRx database. Pharmacoepidemiol Drug Saf. 2013 Mar;22(3):278-85.

Grimaldi-Bensouda L, Rossignol M, Aubrun E, Benichou J, Abenhaim L; the PGRx Study Group. Agreement between patients' self-report and physicians' prescriptions on nonsteroidal anti-inflammatory drugs and other drugs used in musculoskeletal disorders: the international Pharmacoepidemiologic General Research eXtension database. Pharmacoepidemiol Drug Saf. 2012 Jul;21(7):753-759.

Grimaldi-Bensouda L, Rossignol M, Aubrun E, El Kerri N, Benichou J, Abenhaim L; PGRx Study Group. Agreement between patients' self-report and physicians' prescriptions on cardiovascular drug exposure: the PGRx database experience. Pharmacoepidemiol Drug Saf. 2010 Jun;19(6):591-5.