


Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

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Possible role of pandemic AH1N1 swine flu virus in a childhood leukemia cluster


The causation of acute lymphoblastic leukemia (ALL), the main subtype of childhood cancer, is still unresolved and the difficulty in uncovering a likely multi-factorial etiological pathway is compounded by biological heterogeneity of what is a relatively rare disease.1,2

There is persuasive evidence that the major subset of ALL, B-cell precursor or common ALL, is usually initiated pre-natally, in utero and that additional secondary genetic changes more proximal to diagnosis are required to trigger overt disease.2 Any putative etiologic mechanism has to accommodate this pattern of natural history. Among many candidate exposures, infection has long been considered by many as a likely etiologic mechanism has to accommodate this pattern of natural history. A recent (1999–2004), and striking, was in Fallon, Nevada.6

We tested remission DNA of the patients for inherited or constitutive allelic variation in three genes associated with increased risk of B-cell precursor ALL: ARID5B, IKZF1 and CEBPE.7 The number of risk alleles present per case varied from one to five (out of six possible; average three) but does not differ from that expected (Supplementary Table 1).

Figure 1 shows the monthly distribution of cases diagnosed with childhood ALL between January 1999 and September 2011 in Milan; the broad-based peak, determined by two consecutive high frequencies observed in December 2009 (three cases) and January 2010 (four cases), has no equivalent in the considered period of 13 years (see also Supplementary Tables 2 and 3).

Figure 2 shows the results of the Scan statistic for the space–time analysis of ALL cases. The Kulldorff’s scan method8 identified only one significant cluster encompassing six administrative areas in the center of Milan within the time interval from 1 December 2009 to 31 January 2010 (P-value = 0.017) (see also Supplementary Table 4). The cluster is composed of four cases, compared with 0.04 expected, corresponding to a rate ratio of 100.0 (95% CI: 26.9–256.0) for the areas and time period identified. The cases are exactly the four children resident in the surroundings of the school, three of them attending the school.

Because of long-lasting refurbishment, the primary school was not in operation until September 2009; in the previous year, the entire school population was temporarily hosted in the buildings of another school in the same city area. The concentration of all tested physical and chemical agents in the school as well as in the houses of cases did not deviate from normal ranges. Neither parents nor children had noticeable therapeutic exposures to postulated toxicants. None of the parents had professional exposures relevant for leukemia etiology. None of the cases had first-level familial malignancies. (Methods and details in Supplementary).

There was an outbreak of pandemic AH1N1 swine flu virus in a childhood leukemia cluster
children from Milan, 62 (32.3%) were seropositive suggesting the 100% positivity of the seven cases was significant ($P < 0.001$). Five cases of ALL diagnosed before the outbreak were, as expected, all seronegative. Of four solid pediatric tumors (Wilms') diagnosed in Milan following the outbreak, only one was seropositive.

If abnormal immune responses to the widespread pandemic of AH1N1 served as a proximal trigger for ALL in the seven cases, then these cases would have to be ‘susceptibles’. The ‘delayed infection’ hypothesis predicts that B-cell precursor ALL may be triggered by immune response signals but that risk is contingent upon deficits of common infections in infancy that are required to prime the neonatal and infant immune system. This is endorsed by measurement of social contacts in infancy as surrogates for common infections$^{9–11}$ and by modeling in mice.$^{12,13}$

We therefore investigated these characteristics in the seven families involved in the cluster. These were compared with a small series of control cases (Supplementary Table 6). Six out of seven cases were first born compared with nine out of 14 for the controls. All seven cases had parents of medium to high socio-economic class compared with only two of the 20 controls. None of the seven cases attended day care in the first year of life (except for just 1 week in one case). Two of the seven cases attended day care in the 2–3 years of life compared with 8 out of 14 controls. Of note is the related fact that the school with three cases serves an inner area of Milan of high socio-economic status professional parents.

A plausible explanation for the time/space cluster in Milan is that the AH1N1 epidemic provided a strong immunological or...
inflammatory signal that triggered conversion to clinical ALL in a small number of individuals harboring clinically silent pre-leukemic clones but who also were at risk of abnormal response to AH1N1 because of a prior deficit of microbial exposures in infancy that prime or modulate the naive immune system network.²

However, the causal explanation for the cluster we propose is based on associations and we cannot formally exclude a role for chance alone. It is therefore tentative. We encourage other investigators to scrutinize incidence data, both nationally and at local levels or within school systems, to see if other increases in incidence occurred shortly after the 2009 pandemic of AH1N1.

We do not suggest that AH1N1 has a unique etiological role in childhood ALL or that it poses a public health concern. ALL will, at most, be an extremely rare consequence of AH1N1 infection as part of a multi-factorial etiology. We would anticipate that other rare space/time clusters of childhood ALL may also be due to a promotional effect of infectious agents but not to AH1N1. Influenza viruses may, however, merit further study as indirect, promotional agents for ALL. A prior study noted an association between epidemics of seasonal influenza in the UK and significant increases in the incidence of ALL that follow very shortly (~6 months) thereafter.¹⁴ AH1N1, other influenza viruses and, possibly, other viral or bacterial infections may act as triggers or promoters of ALL.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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ETHICS APPROVAL
Written informed consent has been obtained from patients’ guardians; this includes, without limitation, publication in all formats.

AUTHOR CONTRIBUTIONS
GC supervised the biological studies, contributed in collection, analysis and interpretation of the data, as well as in co-writing the paper. LB supervised the comprehensive investigation plan and the cluster analyses, contributed in collecting and interpreting data, and in writing the paper. GR and SD participated to the investigation plan and performed the cluster analyses. SB performed the biological analyses. FP provided the virological analyses and comprehensive investigation plan and the cluster analyses, contributed in collection, analysis and interpretation of the data. MG supervised the biological studies, contributed in collection, analysis and interpretation of the data and co-wrote the manuscript. The corresponding author confirms that he had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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