TABLE I. Between- and within-study group changes in measured variables

Variables	Within-group change across visits* Placebo (95% CI)‡	*P* value	Mepolizumab (95% CI)‡	*P* value	Between-group change across visits (95% CI)†	*P* value
Blood eosinophils§	0.77 (0.58-1.03)	.173	4.6 (2.6-7.9)	.001	6.1 (3.9-9.7)	<.001
% Sputum eosinophils§	1.2 (0.6-2.2)	.347	2.2 (0.6-4.3)	.236	1.8 (1.1-3.0)	.032
FENO§	0.95 (0.7-1.2)	.852	0.96 (0.8-1.2)	.781	1.0 (0.8-1.3)	.924
Postbronchodilator FEV_1/% predicted	0.92 (−2.1 to 3.9)	.172	−0.99 (−6.9 to 4.9)	.456	−1.9 (−6.0 to 2.2)	.433
Modified JACQ	0.05 (−0.2 to 0.3)	.171	0.59 (0.3-0.9)	.001	0.54 (0.01-1.06)	.047

*Within-group changes presented are the mean change for each variable over a 12-month observation period, from the baseline visit of the observation period (ie, final treatment visit of clinical trial), computed by using a repeated-measures model (see the Online Repository at www.jacionline.org).
†Between-group changes are expressed as the within-group change in the mepolizumab group compared with the within-group change in the placebo group. For variables reporting fold change, comparisons of between-group change are expressed as the ratio of fold change.
‡Former allocation treatment in the blinded study. Subjects were treated with standard of care during the follow-up observation period.
§Changes expressed as fold change. *FENO*, Fraction of exhaled nitric oxide.

in patients with severe eosinophilic asthma and recurrent exacerbations.

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Ash pollen immunoproteomics: Identification, immunologic characterization, and sequencing of 6 new allergens

To the Editor:

Ash (*Fraxinus excelsior*) is widely distributed in Northern and Central Europe. Although yearly ash pollen counts are in the range of 1000 grains/m^3, their clinical relevance has been undervalued because ash pollination overlaps with that of Betulaceae.[1] Subsequently, ash has not been included in diagnostic assays,[2] although it is responsible for extensive pollinosis in Alsace (France) and Austria where ash pollination occurs after birch,[2] and in Switzerland where it is as frequent as birch pollinosis.[3]

Allergic patients are commonly diagnosed and treated with protein extracts from natural sources. An alternative approach for allergy diagnosis and personalized treatment of patients consists of the use of purified allergens. For this purpose, it is necessary to know the near complete allergogram of the source responsible for the sensitization.

Despite the complexity of the IgE-reactive protein profile observed by 1D-electrophoresis (1DE) analysis of *F excelsior* pollen,[4,5] Fra e 1 is the only ash pollen allergen isolated, produced as recombinant protein, that has been characterized.[4] We therefore performed an extensive 2D-electrophoresis (2DE)

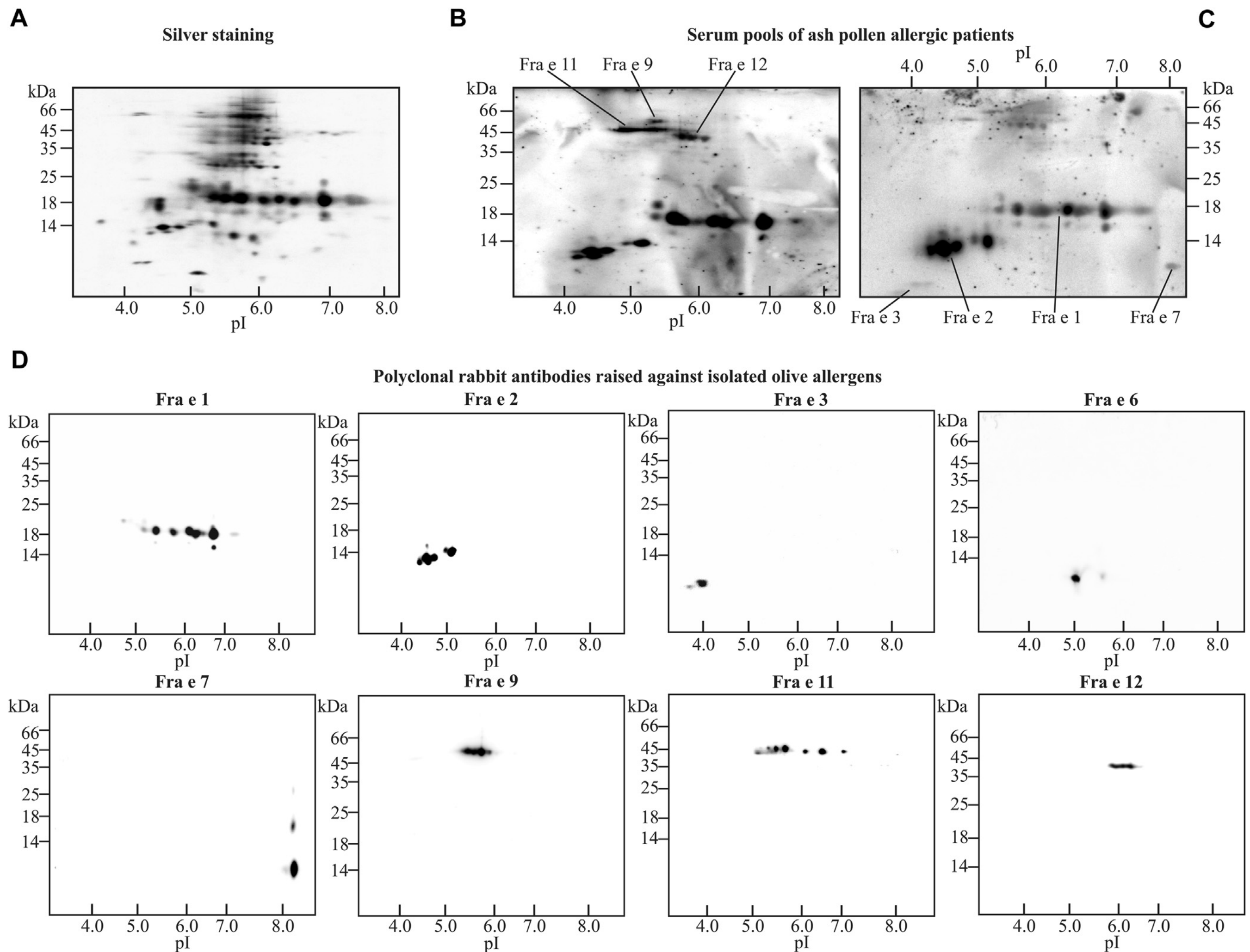

FIG 1. IgE-reactive proteins identified by immunoproteomic profiling of ash pollen extract. **A,** 2DE separation of ash pollen extract followed by silver staining. IgE-immunoblot after 2DE using 15% **(B)** or 17% **(C)** polyacrilamide gels. IgE immunostaining was performed with a selected equivolumetric pool of 8 sera directed against high-MW allergens (Fig 1, *B*) or 8 sera directed against low-MW allergens (Fig 1, *C*).[4,5] **D,** 2DE as in *B* and *C*, and immunostaining with the indicated polyclonal antisera raised against known olive pollen allergens to identify the main IgE-reactive components of ash pollen extract. The IgE-reactive spots named in *B* and *C* were identified according to the IgG reactivity in *D*.

analysis to identify the IgE-reactive protein content by using serum of 25 ash pollen–sensitized patients with IgE reactivity to ash and olive pollen extracts as assessed by ELISA (see Table E1 in this article's Online Repository at www.jacionline.org). On average, silver-stained gels showed 165 ± 15 spots between isoelectric points (pI) 3.5 and 8.2 and molecular weights (MWs) between 4 kDa and 70 kDa (see Fig 1, *A*). To visualize IgE-binding proteins, 2D-immunoblots were performed by using 2 independent pools containing the sera of 8 different ash pollen–allergic patients (see Fig 1, *B* and *C*). We found 35 ± 2 IgE-binding proteins with pIs ranging from 3.8 to 8.1 and MWs ranging between 8 kDa and 66 kDa. A main group of IgE spots centered at mildly acidic-neutral pI and having MWs about 18 to 22 kDa corresponded to Fra e 1 isoforms.[4,5]

We hypothesized that most of the observed IgE-reactive bands might correspond to proteins with similar molecular features (pI and MW) to previously described olive pollen allergens: Ole e 1, Ole e 2, Ole e 3, Ole e 6, Ole e 7, Ole e 9, Ole e 10, Ole e 11, and Ole e 12.[6] To address this question, we then performed a complete immunoproteomic study of ash pollen extract by 2DE by using polyclonal antisera raised against the most relevant olive pollen allergens (see Fig 1, *D*). We identified IgG-reactive proteins and named them as Fra e 1, Fra e 2, Fra e 3, Fra e 6, Fra e 7, Fra e 9, Fra e 11, and Fra e 12, according to the olive homologues recognized by the rabbit antisera. The so identified proteins and their cross-reactive isoforms corresponded to most of the identified IgE-reactive allergens (see Fig 1, *B* and *C*). Fra e 10 was detected only by 1DE (data not shown).

These findings suggest high sequence identity between olive and ash pollen allergens. To confirm this hypothesis, cDNA encoding 6 ash pollen allergens not previously reported (Fra e 2, Fra e 3, Fra e 6, Fra e 10, Fra e 11, and Fra e 12) was amplified by PCR, cloned, and sequenced (see Table E2 in this article's Online Repository at www.jacionline.org).[4] cDNA encoding the

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